# Tilapia Aquaculture in Urban "Brownfields":

# A Demonstration Project

FINAL PROJECT REPORT: AGRAQUA-298

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Prepared for

The Massachusetts Aquaculture Grants Program

Massachusetts Department of Food and Agriculture.

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## PREFACE

### The Massachusetts Aquaculture Grants Program (MAG)

The Commonwealth of Massachusetts has taken numerous proactive steps toward the development of fish and shellfish farming in the Bay State. Commencing in 1994 with a gubernatorial directive to examine the constraints to aquaculture development in Massachusetts, the aquaculture development program at the Massachusetts Department of Food and Agriculture has emerged as a multifaceted program that is important to the continued evolution of the aquatic cultivation industries in Massachusetts. To that end, the development of the Massachusetts Aquaculture White Paper and Strategic Plan generated 68 recommendations that address regulatory, economic development and environmental issues associated with Massachusetts' aquaculture development. Among the Plan's comprehensive recommendations, a variety of grants programs were suggested as means to accelerate industry development through increased public awareness and education, enhanced research and development and improved opportunities for the transfer of relevant methods and technologies.

In 1997, as a result of the efforts of the Massachusetts State Legislature, the Governor's Office, the Executive Office of Environmental Affairs and the Massachusetts Department of Food and Agriculture, the Massachusetts Aquaculture Grants Program (MAG) was created in response to the Commonwealth's interest in aquaculture development and the great need for diversification of fisheries and agricultural enterprises in

Massachusetts. The MAG program encourages environmentally responsible aquaculture projects that can demonstrate public and industry benefit through work that will: result in the development and implementation of new technologies, products, processes or services; reduce aquaculture industry operating costs thereby increasing business profitability; increase the productivity of Massachusetts aquatic cultivation endeavors; and preserve existing jobs and/or result in new employment opportunities for the Commonwealth of Massachusetts.

During the first year of the MAG program $135,000 supported 6 projects that were proposed to address the criteria specified by the Aquaculture Grants Program.

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**B & D Aquatics (Now SEMAP), UMass Dartmouth, Greater New Bedford Regional Vocational Technical High School and Rowland Institute for Science.**

## EXECUTIVE SUMMARY

B&D Aquatics (now SEMAP Inc.), UMass Dartmouth, Greater New Bedford Regional Vocational Technical High School, and others collaborated in a study to evaluate the feasibility of growing tilapia commercially in an urban setting.

The primary project goals were: 1) to evaluate the feasibility of raising finfish in an unused urban warehouse (especially above the ground floor where most urban floor space is available); 2) to demonstrate that existing intensive re-circulating systems technology could be adapted to this setting; 3) to encourage cooperation between the education, government and business communities, and; 4) to develop a program in urban schools to promote understanding and generate interest in aquaculture.

The study also addressed issues such as marketing and distribution and sold a token 1,000 pounds of live fish to wholesalers at $2 a pound. A business plan was developed that addressed the particular regulatory and other requirements of doing aquaculture in Massachusetts. In addition, the study addressed some common concerns such as disease control, fry production and high feed costs.

This project was funded by the Massachusetts Department of Food and Agriculture through the Department's newly created Massachusetts Aquaculture Grants Program with matching funds by the participants and others.

## Tilapia Aquaculture in Urban "Brownfields":

## A Demonstration Project

### Project Introduction

There is public and private interest among entrepreneurs, educators, and public officials in developing inland urban aquaculture in Massachusetts. The fishing industry is undergoing a transitional period and displacing workers who have a keen interest in fishing and in producing fishery products. Public officials are concerned about the pattern of older urban buildings left vacant and in time, often abandoned. Taken together, entrepreneurs see opportunity in this industry because of the "Brownfield" buildings and vacant or underutilized manufacturing spaces available, the proximity to the northeast markets, the long Massachusetts tradition and skill in handling and marketing fishery products, and the strong demand for seafood at a time of decreased fish landings. Educators are interested in aquaculture as a way of arousing curiosity, motivating students, teaching math and science, and teaching problem solving for K-12 and college students. As a profit-making industry it could be used to help revitalize inactive urban sites and create jobs.

Tilapia is one of the finfish species that could make a significant contribution to the aquatic cultivation industry of the Commonwealth of Massachusetts. According to the authors of the Aquaculture White Paper & Strategic Plan, the 1995 tilapia production was insignificant and less than 140,000 pounds (Massachusetts Coastal Zone Management 1995). However, the potential for increased production of this species is well recognized. Maryland was cited as expecting a six-fold increase in production from 250,000 pounds in only two years. The U.S. production in 1995 was 15 million pounds whereas 1998 production is projected to approach 21 million pounds (American Tilapia Association 1998). Even so, this is only incidental to the 62 million pounds live-weight-equivalent the U.S. imported from developing countries in 1996 (Aquaculture Magazine 1997).

Raising tilapia in Massachusetts appears to make sense. Tilapia are African relatives of the 1300 or so species of the Cichlidae family of fishes (Nelson 1994). The approximately 100 species called tilapia are native to sub-Saharan Africa and are intolerant of temperatures below 50oF (Van Gorder and Strange 1981). This makes transporting live fish across state lines less of a concern to environmentalists since accidental release to the wild will not threaten native fish populations or gene pools. Tilapia are hardy, tolerant of low dissolved oxygen, tolerant of low salt water (some species can even survive in full-strength sea water), and they are fecund and easy to breed. Most tilapia species are herbivorous while others are omnivorous and depending on age, require only 24% to 38% protein in their diet for good growth rates. Some tilapia farmers claim 1.1 pound of dry feed will produce one pound of live fish (food conversion ratio, FCR, of 1.1 to 1) and a grow-out time of only 7 months. The average FCR is around 1.4 which is better than that of chicken broiler which averages around 2.0.

Raising tilapia in "Brownfields" also appears to make sense. Acres of vacant manufacturing space exist and tilapia grow best in temperatures of approximately 85oF which can be achieved with indoor recirculating heated tanks. Finally, tilapia aquaculture will not compete with the Massachusetts groundfish fishermen and displace jobs. The market for tilapia is different from the $60 million plus groundfish fishery. Rather, tilapia aquaculture in the U.S. must compete with imported tilapia fillets and frozen whole fish.

A significant constraint on urban tilapia aquaculture reaching its potential is convincing skeptical investors and lending institutions to invest or lend sufficient capital. The skepticism is well founded where existing aquaculture technology would have to be modified and adapted to existing conditions and old buildings in the urban setting. A scale model of a grow-out facility is useful in adapting and refining the intensive recirculating tank technology for growing tilapia in underutilized mill space and having something tangible to show potential investors and lending institutions.

## SEMAP GROW-OUT SYSTEM

### Introduction

In mid-1996, B & D Aquatics, later incorporated and renamed SEMAP, started raising tilapia in the maintenance building at the rear of Cliftex Corporation at 194 Riverside Avenue, New Bedford, Massachusetts (see attached map and Photo C1). A 10,000 gallon circular tank was constructed to serve as the main grow-out tank and juveniles, some bred by SEMAP but most purchased as fingerlings, were stocked in the tank. Three 5,000 gallon rectangular raceways were constructed to serve as nursery and holding tanks to grow fingerlings (around 1") to juvenile size (4 " to 5").

The impetus for B & D Aquatics to grow tilapia was to test the feasibility of aquaculture in unused mill space. Armed with a letter from the largest Asian tilapia buyer in the northeast citing demand for 50,000 pounds of live fish per week at $2/lb, B & D Aquatics undertook the task of raising tilapia in underutilized space in the maintenance building – this with the blessing of one of the previous owners of Cliftex Corporation and the concurrence of the others. The previous owner of Cliftex Corporation who, in partnership with others, owns a 200,000 square foot vacant mill building, was interested in finding productive use for this space (see example of vacant mill space, Photo C2). In addition, B&D Aquatics and the previous owner agreed, in a memorandum of understanding, to allow UMass to use its facilities and space for aquaculture development for UMass' projects, should funding become available. Hence, the sign on the Cliftex maintenance building reads CMAST Urban Aquaculture Development Center.

### The Installation of New Tanks

To test the feasibility of growing tilapia above the ground floor, a two-tank 10,000 gallon circular grow-out system was installed with funding from the MAG program. The tanks were circular, above-ground swimming pools which are relatively inexpensive and easy to install (see Fig. A in

Attachment 1). Because of the limited carrying load of the second floor, each tank can only be filled to a two foot depth, holding 5,000 gallons of water. These two tanks are connected to a common waste filter system. Three more 10,000 gallon tanks were installed on the first floor. Two of these 10,000 gallon tanks are on one filter system and the other has its own filter system. By the end of July 1998, SEMAP had six separate systems with 60,000 gallons grow-out or holding capacity.

Since its beginnings, SEMAP has sold several thousand market size fish at $2/lb, but in June 500 pounds of large tilapia sold for an average of $1.80/lb. The management of SEMAP estimates that they had a total of over 60,000 fish on hand at the end of August 1998, ranging in size from about .5 oz. to 1.5 lb. with a median size of about .4 lb.

SEMAP's current production, however, is far below economically feasible production rates, and SEMAP plans to add 5 more two-tank systems to grow purchased fry to juveniles and add 70 two-tank systems to grow the juveniles to market size. Each tank system will consist of two identical tanks, each 21 feet in diameter with 2 feet of water, which share a filtration and aeration system. As stated above, a prototype of this system has already been installed and is currently operating. See attached report of SEMAP grow-out system.

## Feasibility

Projecting from the limited experiences and expenses of SEMAP, it will be difficult but feasible to raise tilapia profitably in vacant mill buildings. Please see Attachment 1, "Feasibility Study: Executive Summary" and the subsection of this report under separate covers entitled "Feasibility Study for Raising Tilapia in Recirculating Tanks in Massachusetts" by Dan Georgianna.

It is economically feasible to raise tilapia in urban buildings. Selling live and fresh whole tilapia, SEMAP projects gross revenues of $1,469,650 per year and net revenues of $184,526 per year at full production. Assuming that SEMAP borrows capital equipment costs at market rates of interest and uses equity for other start-up costs, the return to equity will rise from -46% in the start-up year to 48% at full production in the fourth year.

Tilapia is an ideal product for Massachusetts for many reasons: high per capita fish consumption, labor skilled in handling fish, and available space at low cost. The limiting factor for production is the size of the market, which is currently quite small. Annual U.S. production of tilapia is around 20 million lb. per year. Assuming that U.S. production doubles over the next 5 years, and that Massachusetts secures 1/4 to 1/2 of this increase, increased employment from tilapia in Massachusetts would range from 50 to 100 people.

## High Feed Cost, Fry Production, and Disease Software

Experiments testing clam belly by-products from the local seafood processing industry as a substitute for expensive fishmeal in tilapia feed is promising. Clam belly is low in ascorbic acid, calcium, phosphorus, and one essential amino acid. These ingredients can be added to formulate a diet. Clam belly added to pellets appears to be a taste enhancer to the fish. See Attachment 3, Feed Study: Clam Belly in Lieu of Fishmeal and Attachment 7, Taste Discrimination Experiments at Rowland Institute.

Producing one's own fry is advantageous to reduce fry cost, to engage in a genetic selection program, and to reduce the risk of importing fry with diseases. See Attachment 5, Tilapia Reproduction and Fry Production Experiments.

Two disease software packages were evaluated. Fish Vet 2 cost $999 and comes on CD, while the Hawaiian Aquaculture Multiple Expert System (HAMES) from the University of Hawaii Extension Service is available free through the internet at the American Tilapia Association website. Fish Vet 2 has graphic interactive features and is extensive in coverage but falls short of its advertised promises and is not very useful for the tilapia farmer. The program is protected by a hardware device that attaches to the printer port. HAMES is a text-based system and offers practical suggestions to problems based on information supplied by the user. HAMES can actually be quite useful. See Attachment 6, Evaluation of Disease Software.

## Greater New Bedford Regional Vocational Technical High School

## Aquaculture Educational Outreach Program

A few years ago, Voc-Tech started aquaculture to provide fresh fish for their Culinary Arts Program. Interest in aquaculture was surprising and the science of fish farming was incorporated into the school's science and math curriculum. The regional schools in conjunction with the PALMS program saw an educational opportunity and requested that Voc-Tech lend its new found expertise in aquaculture and share its resources by holding workshops and by providing and setting up aquarium systems in the schools. Voc-Tech has placed approximately 42 tanks in area grammar, junior, and high schools in the region and in the last year has hosted approximately 3000 visitors on tour of its aquaculture facility. See Attachment 4, Voc-Tech Aquaculture Education Outreach Program.

## Literature Cited

Nelson, Joseph S., 1994. Fishes of the world. 3rd Ed. John Wiley & Sons, Inc., New York.

Van Gorder, S., and Strange, D. J., 1981. Tilapia: Production and Spawning Methods. 11, Aquaculture Project. Organic Gardening and Farming Research Center. Rodale Press, Inc.

## CONCLUSIONS AND RECOMMENDATIONS

**I. Feasibility of tilapia aquaculture in urban brownfields.**

We believe that:

• It is technically and economically feasible to raise tilapia in vacant mill space.

• The market for tilapia is thin and needs to be broadened.

• A successful tilapia business requires an entrepreneur with faith and commitment and, most important, adequate resources to weather set-backs.

• Once a business establishes a system for growing tilapia, diversification into other areas, including hydroponics, is recommended.

## aquaculture in MASSACHUSETTS

There is little doubt that fish farming will eventually be financially successful. The current regime of high prices for native species and their products will certainly remain, because almost all commercial fish stocks are currently fished at or above their maximum yield. While there is great debate over the causes of the current declines in commercial fish stocks, few if any scientists or fishermen expect native stocks to increase.

As with most new industries, there have been more failures than successes in aquaculture. The sharp increases in shrimp, salmon, and catfish production, however, show that aquaculture can be successful.

Successful aquaculture of tilapia, hybrid striped bass, sea bass, or other finfish species requires solutions to several crucial problems, including:

• High feed costs

• Shortages of skilled labor

• High capital costs

• Unpredictable and unsteady markets

• High cost of space

• High energy cost

• High cost of filtration and waste water treatment

**High feed costs:**

As shown in three independent studies of production and capital costs for tilapia, feed cost, at $.25 to $.28 per pound, is the most expensive factor in raising tilapia. Tilapia, like catfish, are herbivores, and as with catfish, successful feed cost reduction will probably focus on substitution of soy and other cheap protean for fish meal. This has already begun, and large-scale production will probably lead to successful formulas for less expensive feed, as has happened with catfish.

The ocean clam processing by-product, clam belly, is very promising as an ingredient supplementing or replacing fishmeal in tilapia and other fish feed. Clam belly is low in ascorbic acid, calcium, phosphorus, and one essential amino acid. These ingredients can be added to formulate a balanced diet. Clam belly added to pellets appears to be a taste enhancer to the fish. Finding a way to cheaply reduce and dry it will probably lead to lower feed cost as well as adding to the profits of the clam processors. **We recommend more research along this line.**

**Shortages of skilled labor:**

Tilapia and other finfish raised in recirculating tanks require trained labor. Fishing ports like New Bedford have a natural advantage over other areas because reductions in stocks and fishing effort have made thousands of fishermen and fish processors available to work in aquaculture. These men and women are skilled in handling and processing fish and their products.

**High capital costs:**

Raising tilapia and developing markets for high value added products are very risky. As with most risky ventures, investors want a major share of equity in exchange for their capital, which reduces return rates for entrepreneurs who develop production techniques and markets. **The alternative, which we recommend, calls for debt financing through banks. This will work only if loans are guaranteed by some government agency or other financially secure institution.**

**Unpredictable and unsteady product markets:**

Live fish and fresh fillets are the only profitable markets for tilapia raised in tanks in the Northeast. These markets are extremely thin, i.e., sharp or sudden increases in supply quickly depress prices. Also, buyers are often financially unreliable or haggle over prices after they receive delivery. The supplier who has already shipped fresh or live product finds himself in a difficult situation when haggling over prices. The market needs to be broadened and the tilapia farmer needs to diversify into other species. Once the details of the tilapia farmer's system for raising tilapia is reasonably well worked out, diversification into raising other species should be initiated to accommodate market demands and to smooth out income flow.

**High cost of space and high energy cost:**

At this point of development, raising tilapia in tanks in the Northeast requires very inexpensive space. Suppliers need long term, secure space at low prices in order to concentrate their activity and resources on problems concerning raising and selling their product. The same is true for electrical costs. Although a relatively small factor in cost relative to feed, suppliers need secure, long term, and low cost energy.

**High cost filtration and waste water treatment:**

The density of fish and growth rate in a recirculating system is limited by the capacity of the filtration system to remove fish waste and uneaten food. A low cost filtration system is needed to make a profit. Because of the upward farmgate price limit of $2/lb for live fish, a grow-out system target capital cost of $1/gallon rather than $10/gallon is needed. Further, at full production, disposal of waste water becomes a factor. On-site pre-treatment of the water is necessary for water recycling or disposal into the municipal system.

**Public-private partnership:**

Public-private partnership and cooperation is necessary to fully develop this industry. The MAG program is a small but positive step in this direction. **We recommend the continuation and expansion of the MAG and other such programs.**

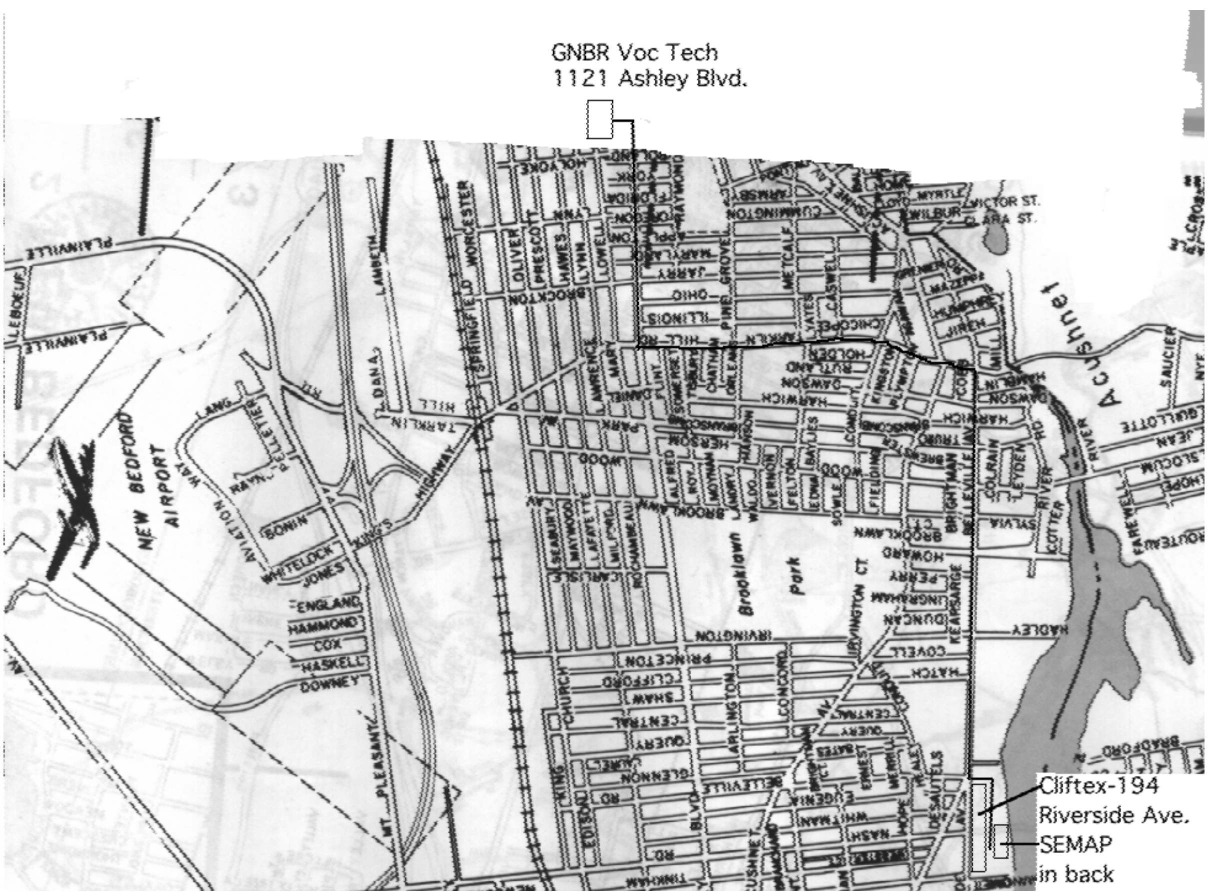
For example, the South has research and co-op programs for catfish. Catfish research is going on at the Department of Fisheries and Allied Aquaculture at Auburn University, the Cooperative Extension Program at the University of Kentucky, Delta Research and Extension Center, and Mississippi State University. Cornell University has The Cornell Aquaculture Program.

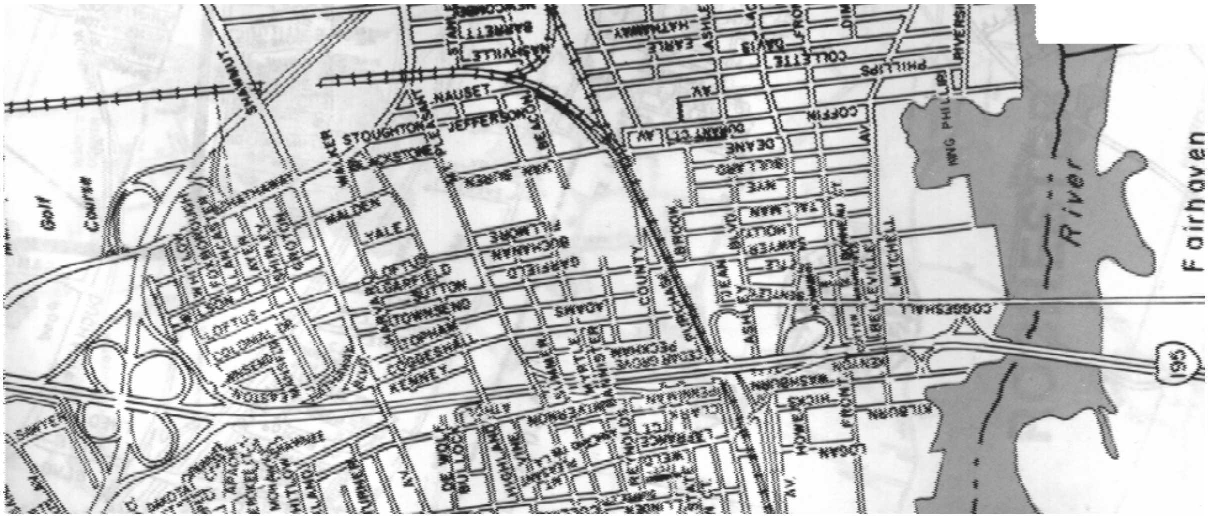
**II. Voc-Tech Aquaculture-Educational Outreach Program.**

Voc-Tech Aquaculture-Educational Outreach program has been very popular with regional K-12 schools. Such programs encourage the sharing of resources and cooperation among the public schools. Voc-Tech has worked with and shared resources with UMass as well. Several joint experiments have been conducted collaboratively with students and resources from both institutions participating and contributing in the studies, for example, Chitosan I, the effects of chitosan on water quality and growth of tilapia; Chitosan II, clam bellies as a protein source in lieu of fishmeal in tilapia feed; Brownfields, fry production experiments; and Brownfields, calcium and phosphorus supplements to clam belly pellets. **We recommend that the Voc-Tech outreach program continue to be encouraged and supported.**

Map from GNBR Voc-Tech to SEMAP.

Turn right onto Ashley Blvd., at traffic light turn left onto Tarkiln Hill Rd., two streets beyond Acushnet Ave. turn left onto Belleville Ave. to SEMAP.






Map to SEMAP from 195 heading east. Take Washburn Exit, turn right and another right onto Belleville Ave. to SEMAP.

**ATTACHMENT – 1**

**Executive Summary Only* of:**

## Feasibility study for

## Raising Tilapia in Recirculating Tanks in Massachusetts

**By Daniel Georgianna, Ph. D., fishery economist**

**August 24, 1998**

A Subsection Of The Project Report: AGRAQUA-298

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*See full feasibility report under separate covers.

## Executive Summary of Feasibility Study

**By Dan Georgianna**

Tilapia are hardy fish that can tolerate high density with the accompanying low oxygen and high ammonia concentrations. Tilapia also exhibit high resistant to disease and favorable feed conversion ratios (lbs. of feed/lb. gained), usually around 1.4, better than conversion ratios for chicken broilers, which average around 2.0. Tilapia has been grown in ponds in warm countries in Africa and Asia for thousands of years, and more recently in raceways and tanks in northeastern sections of the U.S. While growing tilapia in indoor tanks entails higher energy cost, transportation costs to market are lower, because most markets for tilapia are in the large cities of the northeast. Growing tilapia in tanks also give growers more control over the environment and allows higher densities. Some investigators expect the intensive aquaculture of tilapia to follow the highly successful pattern of farmers moving from outdoor farming to confinement for chickens during the late 1950s and early 1960s, and for turkeys during the 1980s. Poultry farmers increased productivity by 1,000 percent between 1951 and 1991 through intensive, indoor farming.

This feasibility study estimates costs for raising tilapia from purchased fry to market size using two estimations from the literature and an estimation from SEMAP, a Massachusetts producer of tilapia. SE Mass Aquaculture Products, Inc. (SEMAP) has been producing live and fresh/whole tilapia for the past 2 years on an experimental basis in New Bedford mill space as a privately funded operation with some funding from a grant from the Massachusetts Aquaculture Grants Program. Currently, SEMAP has about 60,000 fish on hand, ranging in size from about 1/2 oz. to 1 1/2 lb. with a median size of about 1/2 lb. contained in six separate systems with 60,000 gal. grow-out or holding capacity. Over the past year SEMAP has sold several thousand market size fish with about 500 pounds of large tilapia sold in the last month.

Production of tilapia exhibit strong economies of scale, i.e., cost per unit fall as production increases. Losordo and Westerman, using computer simulation estimate capital costs of $0.43 per lb. and operating costs of $0.84 per pound for a small operation producing 100,000 lb. per year. Timmons using data from an experimental operation at Cornell University and a large scale private operation near the University estimate capital costs of $0.13 per lb. and operating costs of $0.61 per pound for a larger operation producing 1.3 million lb. per year. SEMAP estimates capital costs of $0.25 and operating costs of $0.82 for an operation of 1.2 million lb. per year.

Selling live and fresh/whole tilapia, SEMAP expects gross revenues of $1,469,650 per year and net revenues of $184,526 per year at full production. Assuming that SEMAP borrows capital equipment costs at market rates of interest and uses equity for other start-up costs, the return to equity will rise from -46% in the first or start-up year to 48% at full production in the fourth year.

Massachusetts has many factors that make tilapia an ideal product: high per capita fish consumption, labor skilled in handling fish, and available space at low cost, but the limiting factor for production is the size of the market, which is currently quite small. Annual U.S. production of tilapia is around 20 million lb. per year. Assuming that U.S. production doubles over the next 5 years, and that Massachusetts secures 1/4 to 1/2 of this increase, increased employment from tilapia in Massachusetts would range from 50 to 100 people.

**Attachment 2**

# SEMAP Grow-Out System

**By James Tassinari, Eric Stone and Richard M. Ibara**

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## SEMAP Grow-Out System

### Introduction

This section is a description of the grow-out system used at Southeastern Massachusetts Aquaculture Products (SEMAP) formerly known as B & D Aquatics.

Different system technologies have been explored during the past two years at SEMAP with some components proving to be advantageous in this mill setting. A recirculating system with minimal water exchange is necessary at SEMAP due to the high cost of energy to heat the water. A recirculating system allows water to be reused over and over again with less than a 10% daily turnover. The key to a successful recirculating system is its capacity to remove the waste from the fishes and from uneaten food. To maximize profitability in an aquaculture operation, fish must be fed at or near their maximum rate of consumption to ensure rapid growth to market size. However, as fish metabolize the feed, their bodies create both solid and dissolved ammonia waste. If the system can not remove the waste as quickly as it is created, harmful to lethal conditions can develop in the tank resulting in serious problems for the aquaculturist. Poor water conditions can cause stress to most species of fish which inhibits their ability to grow and fend off disease. Therefore, it is vitally important to select a system that will allow fish to eat and grow at their maximum rate while maintaining healthy water conditions. The following is a description of the components used at SEMAP.

### Tanks

The current operation at SEMAP began with one ten thousand gallon above-ground swimming pool as the grow-out tank. Although the round tank was generally successful, it was postulated that rectangular tanks would improve floor space use and make grading and harvesting an easier chore. Three custom 5,000 gallon rectangular raceways were designed and constructed out of lumber and plastic liners. The tanks did allow easier grading of the fish, but the fish did not thrive in these tanks. The shape of the tanks inhibited removal of solid wastes and did not allow the fish to swim against the current as was observed in the round tank. When further expansion at SEMAP took place, it was decided that the inexpensive, easy to install swimming pools were a better choice in this situation (Fig. A).

### Waste Solids Removal

The first stage of water treatment in a recirculating system is solid waste removal. Waste solids in the form of fecal material and uneaten feed pellets need to be removed as quickly as possible. If left in the system, heterotrophic bacteria will multiply rapidly, consume valuable oxygen needed for the fish, and release toxic ammonia as they feed on the waste. Waste solids can be classified as settleable and non-settleable types (see Losordo 1997, for general discussion of recirculating systems engineering).

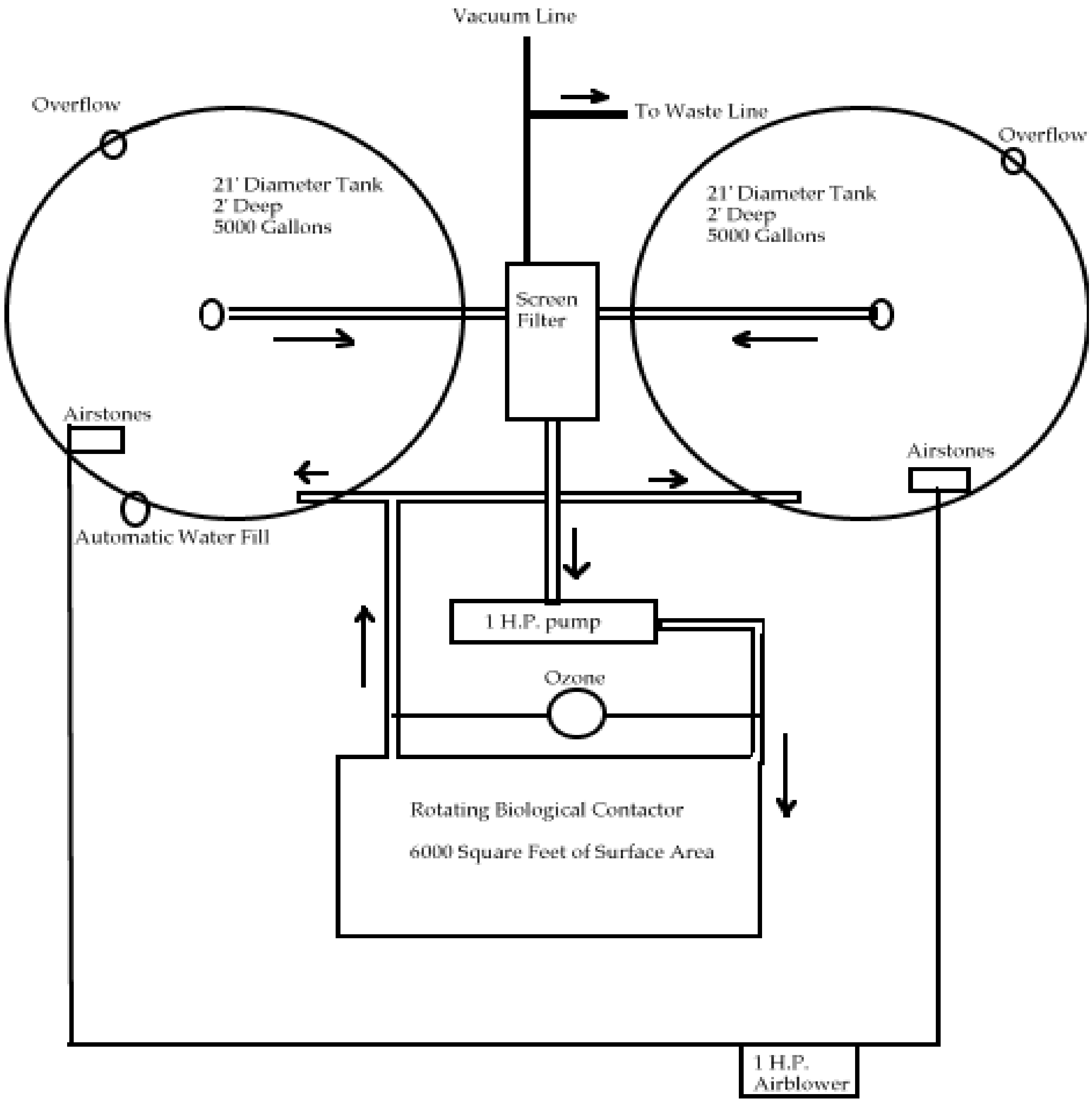



Fig. A. Diagram of second floor 10,000 gallon two-tank system.

Settleable solids are generally larger in size and will settle out of the water within one hour. At SEMAP, two types of screen filters have been used to remove settleable solids. Neither is without disadvantage. Vacuum screen filters are constructed with stainless steel mesh cylinders that rotate within waste laden water pumped from the bottom of the tank. The waste attaches to the exterior of the screen, and allows the cleaner water to continue to the next filtration stage. A vacuum sucks the solids off of the screen as it emerges from the water due to the rotation created by a small motor. The advantage of this filter is that very little water is lost from the system, less than 2% of the volume entering the filter. However, this type of filter is costly both to buy and operate. Typically these filters cost about two thousand dollars each and use a relatively large amount of electricity to run the vacuum. As with anything that has many moving parts, it is prone to break down.

The second type of screen filter used at SEMAP incorporated a high pressure stream of water to spray the waste from a motor-driven rotating plastic mesh cylinder into a waste channel. Although cheaper to buy, this type of filter was costly in terms of water usage. The water used to rinse the mesh had to be clean and excessive amounts were needed to run the filter on a continuous basis. The spray was not as effective at removing the waste as a vacuum and this led to clogging and algal growth. Problems with the bearings on this filter contributed to its unreliability. Improving settleable waste solid removal is an area that would be of great benefit to SEMAP in the future.

Non-settleable wastes, or suspended solids, generally will not settle out of the water column within an hour in calm conditions. These wastes can irritate the gills of the fish as well as increase the biological oxygen demand (BOD) of the system. At SEMAP, a foam fractionator (AKA a protein skimmer) is used for the removal of non-settleable wastes. A foam fractionator works by bubbling air or ozone up through a closed column of water. As the bubbles rise, solid particles attach to the bubbles surface creating a foam which is then channeled off at the top of the column. A sand filter commonly used for swimming pools was also put into use at SEMAP. However, this type of filter quickly clogs with waste and requires frequent backwashing, which wastes a large volume of water.

**Nitrogen Control**

As fish consume their feed pellets, the digestion of the protein within the pellets yields nitrogenous compounds that can be toxic to the fish. In contrast, carbohydrates do not contain nitrogen and lipids have insignificant amounts in them. In a recirculating aquaculture system, where water exchange is minimal, these compounds can concentrate to lethal levels. These compounds, easily measured as total ammonia-nitrogen or TAN, consist of the highly toxic un-ionized ammonia ($NH_3$) and non-toxic ionized ammonium ($NH_4$+). The ratio of toxic $NH_3$ to non-toxic $NH_{4+}$ depends chiefly on the pH and temperature of the water. When the pH and temperature of the water is elevated, this increases the percentage of $NH_3$ in the water. Biofiltration is one of the cheapest way by which harmful ammonia and nitrite are converted to non-toxic nitrate by nitrifying bacteria. These bacteria, *Nitrosomonas*, which converts ammonia to

nitrite, and *Nitrobacter*, which converts nitrite to nitrate, require a suitable substrate on which to attach and favorable growing conditions, including high amounts of oxygen.

Four different methods of biofiltration have been attempted at SEMAP. The first method is commonly referred to as a trickling filter. The trickling filter at SEMAP uses shredded plastic as the substrate to which the bacteria attach. Water is passed through a rotating sprayer onto the plastic media where it slowly trickles to the bottom of the filter. The bacteria attached to the plastic convert the ammonia and nitrite to nitrate and the water then returns to the tank. This method required no additional oxygenation but the holes in the sprayer frequently clogg causing the media to dry out thus killing the beneficial bacteria.

The second method incorporated the same shredded plastic, but this time the media was submerged and air was bubbled up through the filter. This type of filter was successful but doubts raised about its filtering capacity caused SEMAP to explore other filtering options.

A floating bead filter that utilized plastic beads in an enclosed column was touted as having the ability to serve double duty as both a suspended solid removal system as well as for biofiltration. However, this filter did not work effectively at SEMAP. The neutrally buoyant plastic balls that were supposed to remain within the filter would no longer be neutrally buoyant when the waste attached to it. This caused the balls to find their way out of the filter and into the rest of the system. Constant cleaning of the balls is needed for this system to work.

A rotating biological contactor (RBC) was the next biofilter tested at SEMAP. This RBC consisted of corrugated plastic cut into circles and stacked to form a cylindrical honeycomb type of filter. This cylinder rotates through the treatment water due to the lifting action from the aeration on one side of the wheel. The bacteria attached to the plastic substrate are alternately exposed to the water and high amounts of oxygen in air as the cylinder rotates. This type of filter is very expensive when compared to the other filters, and is not cost effective. Many problems were encountered when using this filter. As the bacteria grew, the weight of the wheel increased causing it to jam often. A brief 30 minute power outage at SEMAP created an imbalance in the bacterial growth on the cylinder which took six weeks to correct. The moving parts of the RBC were also prone to frequent breakdown.

Thus far, the submerged shredded plastic biofilter has proven to be the most cost-efficient and effective of the biofilters tested.

**Total Ammonia Nitrogen (TAN) Experienced at SEMAP**

SEMAP experienced a few alarming periods of extremely high levels of TAN. One of the most notable was in late January when the biological filters crashed simultaneously in four separate Tanks – A, B, C, and D (Fig. 1). The shredded plastic in Tanks B, C, and D turned from brown to white with the death and shedding of the bacterial colony from the fibers. The RBC of Tank A shredded the cells and brown

bacterial scum, turned white, and floated higher in the water. The time of the crash corresponded to switching of feed and may be linked to it. But the crash is more likely due to some chemical in the municipal water supply, probably a pulse of chlorine or other disinfectant. This conclusion is consistent with other observations.

With the high level of TAN, especially in Tank A which housed about 10,000 fish averaging 200 g, mortality of one or two fish per day resulted. As an attempt to lower the TAN and toxic ammonia, feeding was reduced, up to 25% of the water was replaced per day resulting in a temperature drop (Fig. 2), and the pH was lowered (Fig. 3). The TAN remained high for several months reaching levels above 10 mg/L (Fig. 1). Reduced feeding helps in reducing ammonia but starved fish will metabolize their muscle mass and produce ammonia. After awhile, it was noticed that changing relatively large volume of water in Tanks B, C, and D caused ammonia levels to peak. Chlorine was tested but was not detectable. Reasoning that replacing the water was the cause of the failure of the biofilters, the activated charcoal filter cartridges were replaced and water exchange was reduced to about 5%/ day. The ammonia level rose briefly in Tank A then drop to under 2 mg/L.

**pH and Alkalinity Control**

As mentioned earlier, the ratio of toxic $NH_3$ to non-toxic $NH_4^+$ depends chiefly on the pH and temperature of the water. When the pH and temperature of the water is elevated, this increases the percentage of $NH_3$ in the water. However, a pH that is too low can decrease the activity of the nitrifying bacteria. Therefore, maintaining a proper pH is very important. Generally it is desirable to maintain the pH of a freshwater system at between 7.0 and 7.5. At SEMAP, baking soda was continuously added to the system to maintain the pH in this range. This also served to maintain the alkalinity of the water which is a measure of its capacity to neutralize acidity. When water is highly alkaline, it is resistant to changes in pH.

**Oxygenation and Disinfection**

Maintaining an adequate dissolved oxygen (DO) level within the water is an extremely important consideration in a recirculating system. An aquaculturist must replenish the water with oxygen at a rate that matches the amount consumed by the fish and bacteria. An important consideration is whether the biofilter is submerged or non-submerged. A non-submerged biofilter can obtain adequate DO from the atmosphere while a submerged biofilter will need to be supplied from the water. Dissolved oxygen is added to the water in one of two ways. Aeration involves exposing a large surface area of the water to air (21% oxygen). This is most efficiently done by bubbling the air through the water. Oxygenation introduces gaseous oxygen (95-100% oxygen) into the system. At SEMAP, both types are utilized. Aeration is accomplished by use of a 1 HP air pump and airstone diffusers within the tank. A down flow bubble contactor (DFBC) is used as a means of introducing gaseous oxygen as well as ozone into the system. The DFBC uses physics to efficiently introduce the oxygen into the water with very little waste. The ozone added to the system acts as disinfectant oxidizer of dissolved organics.

**Waste Management**

While concentrating on ways to remove waste from a recirculating aquaculture system, the aquaculturist must also take into consideration what will be done with the waste once it is removed. Environmental concerns have often inhibited the expansion of aquaculture in the Northeast. Simply dumping the wastes back into the environment can be detrimental to the ecosystem and dumping into municipal sewage treatment systems can be expensive. Future plans at SEMAP include turning the waste into a useful product, thus minimizing treatment costs. At full-scale production (50 or more tanks), it would be economically feasible to create a treatment system that would produce enough waste for a marketable fertilizer and compost product. In this treatment system, further separation of the solids from the water would occur. The water would be sterilized, ozonated, reoxygenated and reintroduced to the system, thus saving water costs. The solids would be treated and sold to defray the operating costs of the system.

**Literature Cited**

Losordo, Thomas M., 1997. Tilapia culture in intensive recirculating systems. In B.A. Costa-Pierce and J.E. Rakocy, eds. Tilapia Aquaculture in the Americas, Vol. 1. World Aquaculture Society, Baton Rouge, Louisiana, United States.

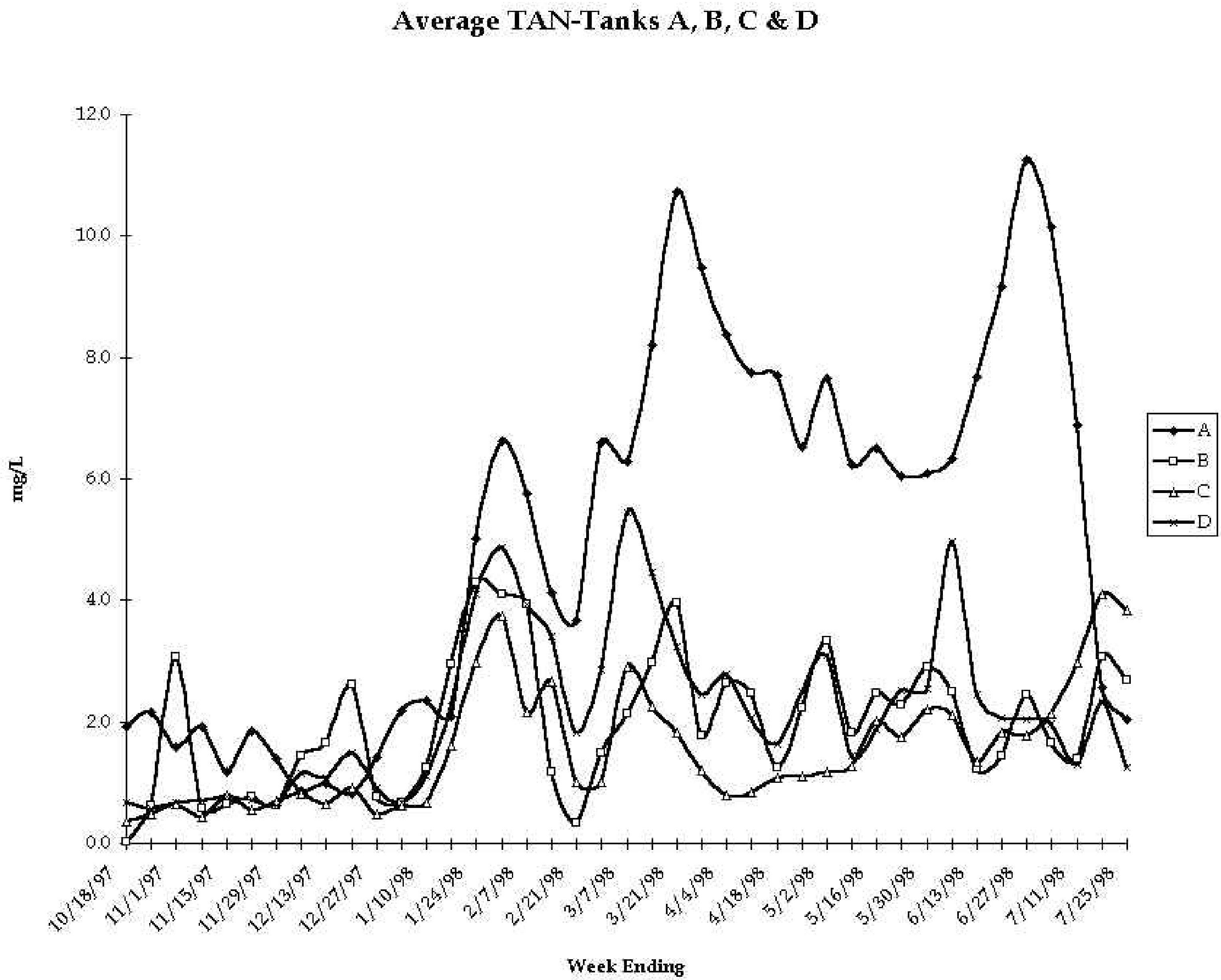
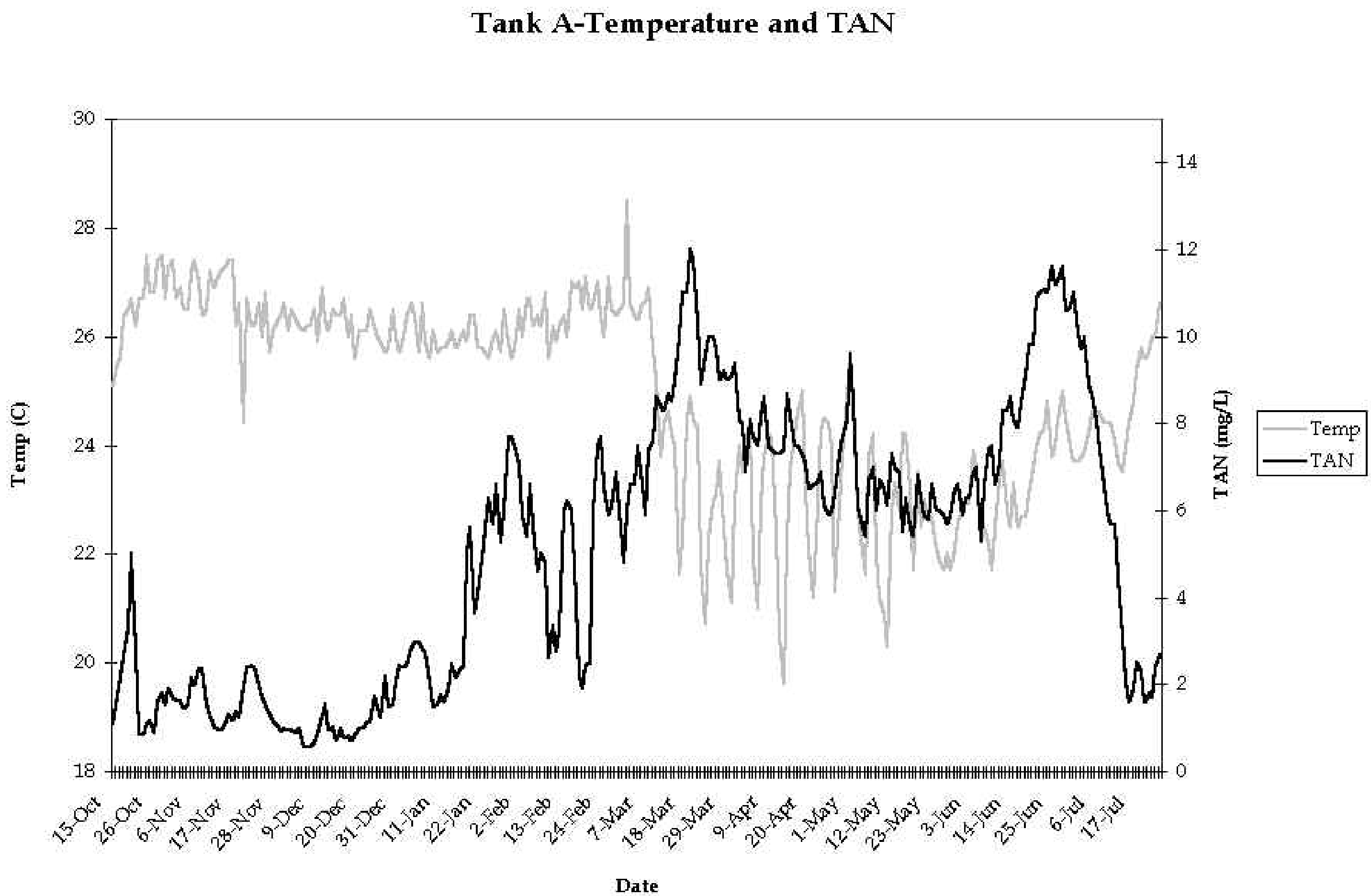
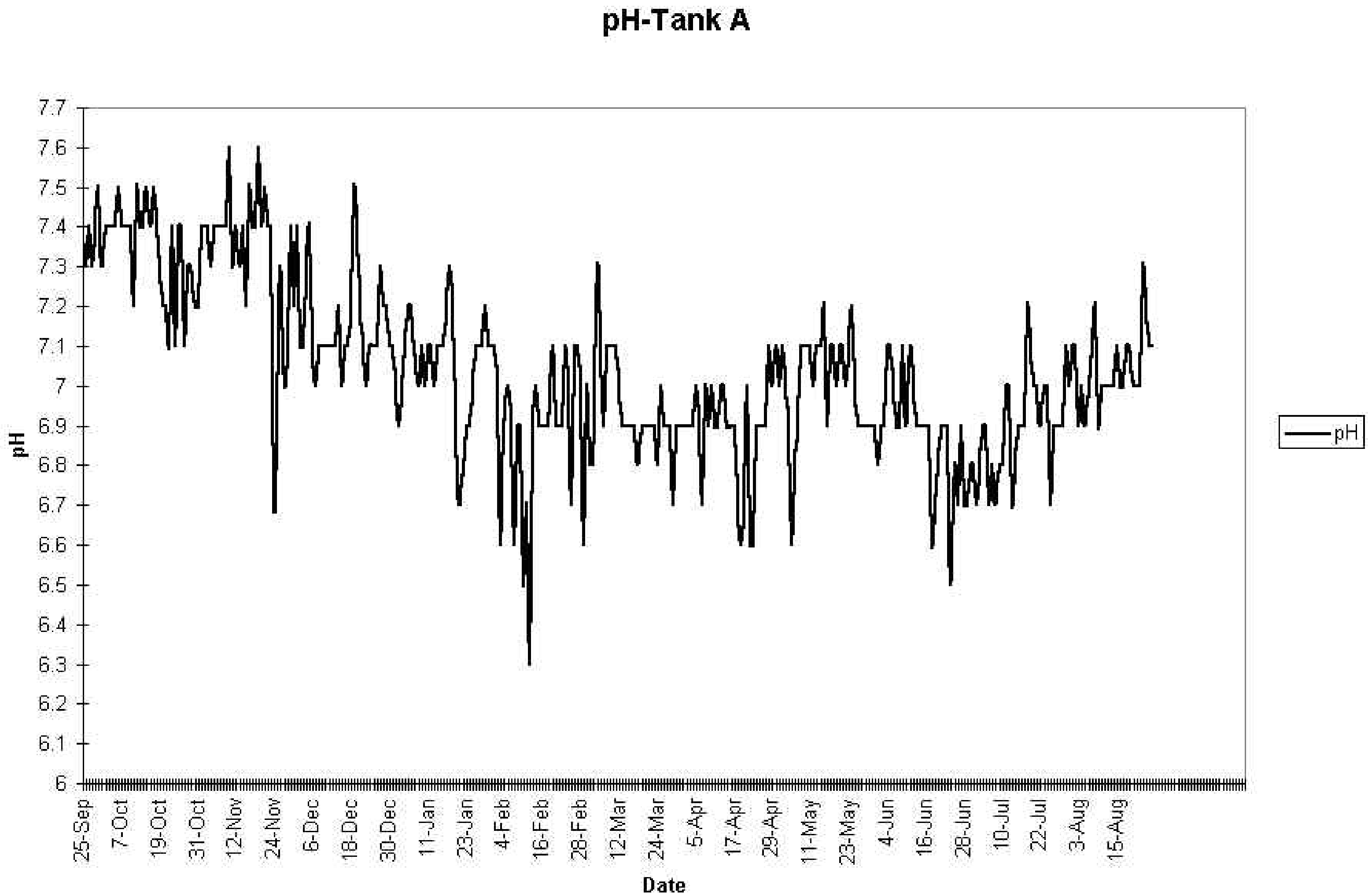

Average TAN-Tanks A, B, C & D
12.0
10.0
8.0
6.0
4.0
2.0
0.0
mg/L
10/18/97
11/1/97
11/15/97
11/29/97
12/13/97
12/27/97
1/10/98
1/24/98
2/7/98
2/21/98
3/7/98
3/21/98
4/4/98
4/18/98
5/2/98
5/16/98
5/30/98
6/13/98
6/27/98
7/11/98
7/25/98
Week Ending
A
B
C
D

FIG. 2 TANK A TEMPERATURE & TAN (SEE ERIC'S GRAPHS, F2) The temperature and Tan graphs might need to be combined.




INSERT FIG. 3 – PH TANK A




**Attachment 3**

**Feed Study: Ocean quahog (*Arctica islandica*) bellies supplemented with phosphorous and calcium as an alternative to fishmeal in the diet of intensively cultured hybrid tilapia (*Oreochromis niloticus x O. aureus*)**

**By Eric Stone and Richard M. Ibara**

A Subsection Of The Project Report: AGRAQUA-298

"Tilapia Aquaculture in Urban "Brownfields":

A Demonstration Project"

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Massachusetts Department of Food and Agriculture.

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## INTRODUCTION

For tilapia aquaculture in Massachusetts to flourish, production costs need to be identified and reduced so that the tilapia farmer can compete successfully with established fish farmers in other regions of the U. S. and overseas. One way to do this is to reduce the price of the feed which can represent 40-60% of a fish farmer's overhead (Rodriguez-Serna et al. 1996). Tilapia feed cost from $.25 to $.28 per pound of product and ranks highest among inputs in raising tilapia (Georgianna 1998). Intensive fish farming currently depends upon the use of fishmeal as the sole or major source of dietary protein and lipid in formulated compound feeds (McCoy 1990). Fishmeal is the most expensive, but the most crucial ingredient in aquaculture feeds. It is composed of whole fish that is cooked, dried and ground into a powder. Five pounds of fish are needed to produce one pound of fishmeal. The finished product is 60-80% protein which is 80-95% digestible by fish (Lovell 1989). Increasing demand from all sectors of agriculture is likely to drive the price of fishmeal higher (McCoy 1990). As a commodity, fishmeal is prone to large fluctuations in price; in 1995 it experienced a dramatic increase (Spatz et al. 1996).

Tilapia are herbivores and successful feed cost reduction will probably focus on substitution of soy and other cheap protein for fish meal. Proteins from different plants have been tested and have shown varying levels of success. Plant products such as soybean meal (Shiau et al. 1990), macadamia presscake (Balogun and Fagbenro 1995), alfalfa leaf (Olvera-Novoa et al. 1990), rapeseed meal (Davies et al. 1990), and cowpea and blackgram seeds (Keembiyehetty and DeSilva 1993) have successfully replaced a portion of the protein in tilapia feed. Animal products have also been used effectively. Fish protein hydrolysates (Berge et al. 1996) and silages (Fagbenro 1994), as well as chicken offal (Balal et al. 1993), have been substituted for a percentage of fishmeal without ill effects. Other successful animal protein alternatives include krill meal (Lou and Chen 1980), blood meal (Otubusin 1987), hydrolyzed feather meal and meat and bone meal (Tacon et al. 1983).

In a previous study, we found that the shredded bellies of Ocean quahogs (*Arctica islandica*), a by-product of clam processing in Massachusetts, could replace 50% of the fishmeal in tilapia feed pellets without adversely affecting growth (Stone 1998). However, when clam bellies replaced 100% of the fishmeal in the feed pellets, appetite and growth became suppressed in the fish after an initial three week period of promising results. Further, fish fed this formulation exhibited skeletal deformities consistent with a mineral deficiency. Analysis of the feed pellets indicated that calcium levels of 0.15% were well below the recommended minimum of 0.65% (Table 1). Experiments conducted at the Rowland Institute revealed that when given a choice between feed pellets composed of the clam bellies and ones made from fishmeal, tilapia preferred the clam belly pellets by a 9:1 ratio.

Table 1. Mineral analysis of the experimental feed pellets used in previous

study (Stone 1998) in parts per hundred (%) and parts per million (ppm).

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Minimum |
|---|---|---|---|---|---|---|
| | FM | HH | CB | ZG | IF | Requirements |
| calcium (Ca)% | 1.43% | 0.69% | **0.15%** | 0.71% | 2.44% | **0.65%**a |
| phosphorus (P)% | 1.12% | 0.66% | 0.28% | 0.92% | 2.08% | .30a to .90%b |
| iron (Fe) ppm | 409 | 204 | 197 | 491 | 453 | 30 to 170c |
| zinc (Zn) ppm | 32 | 31 | 34 | 130 | 239 | 15 to 40c |
| manganese (Mn) ppm | 11 | 9 | 9 | 113 | 71 | 2 to 20c |
| copper (Cu) ppm | 5 | 5 | 7 | 16 | 22 | 1 to 5c |
| selenium (Se) ppm | 4.7 | 3.9 | 4.1 | 4.8 | 8 | .15 to .50c |
| cobalt (Co) ppm | bd | bd | bd | bd | 3.46 | .05 to 1c |
| potassium (K) % | 0.58% | 0.41% | 0.41% | 0.68% | 0.77% | |
| sulfer (S) % | 0.34% | 0.41% | 0.38% | 0.43% | 0.28% | |
| magnesium (Mg) % | 0.14% | 0.12% | 0.11% | 0.22% | 0.32% | |
| silicon (Si) ppm | 170 | 42 | 40 | 15 | 39 | |
| aluminum (Al) ppm | 29 | 45 | 94 | 102 | 199 | |
| arsenic (As) ppm | 2.1 | 2.2 | 3.8 | 3.2 | 3.3 | |
| chromium (Cr) ppm | 2.9 | 2.6 | 2.8 | 3.7 | 6 | |

| | | | | | |
|---|---|---|---|---|---|
| nickel (Ni) ppm | 2 | 2.1 | 2.9 | 2.3 | 3.9 |
| molybdenum (Mo) ppm | 1.1 | 1.4 | 2 | 2.9 | 4.4 |
| vanadium (V) ppm | 0.82 | 0.97 | 1.28 | 1.85 | 3.14 |
| lead (Pb) ppm | 0.84 | 0.86 | 0.46 | 1.71 | 5.75 |
| cadmium (Cd) ppm | 0.61 | 0.6 | 0.63 | 0.41 | 0.61 |
| ytrium (Y) ppm | 0.19 | 0.21 | 0.31 | 0.34 | 0.64 |
| mercury (Hg) ppm | 0.01 | 0.01 | 0.03 | 0.01 | 0.04 |
| bd= below detectable level | | | | | |
| a=(Robinson et al., 1984)(Robinson et al., 1987) | | | | | |
| b=(Watanabe et al., 1980) | | | | | |
| c=(Watanabe et al., 1997) | | | | | |

Ocean quahogs are found in deep waters from Newfoundland to North Carolina and off the coast of Northern Europe. This species supplies an important percentage of marketed clam products (Dore 1991). The meat has a strong flavor and is tough and dark in color which makes it less valuable than the surf clam (*Spisula solidissima*). Ocean quahogs, as a minced product, sell relatively well but only if the bellies are discarded. The dark algae that is the staple diet of this clam severely discolors the minced product. Processors in Rhode Island and New Bedford produce over 80 tons of clam bellies per week. Currently, most of the bellies are composted at a hauling cost to the processors.

Tilapia culture is one of the fastest growing sectors of the aquaculture industry. World production of tilapia doubled between 1986 and 1992 to 473,000 metric tons (FAO 1994). U.S. production in 1994 was approximately 13 million pounds (Aquaculture Magazine 1995) and imports increased 54% from 1993 to 1994 to about 45 million pounds for a total usage rivaling that of trout (USDA 1994). In the Northeast, tilapia

production for 1995 was 580,000 pounds which was 3.6% of U.S. production (Aquaculture Magazine 1995). This represents a 107% increase in production since 1992 (Bush and Anderson 1993). The reason for the increasing popularity of tilapia culture can be traced to their bone-free, high quality flesh, rapid growth and tolerance to disease and low dissolved oxygen (Lovell 1987).

There are over 100 species of fish native to Africa commonly referred to as "tilapias" (Van Gorder and Strange 1981). They are tilapiine fishes represented by three genera, *Oreochromis*, *Sarotherodon* and *Tilapia* as defined by Trewavas (1982 and 1983 in Costa-Pierce and Doyle 1997). *Oreochromis* are maternal mouthbrooders, *Sarotherodon* are paternal mouthbrooders, and *Tilapia* are substrate spawners. Tilapia are members of the Cichlidae family and are typically freshwater fishes although some species are salt tolerant (Avella et al. 1993; Stickney 1986). They are able to withstand water temperatures exceeding 34°C and dissolved oxygen levels of <1 ppm (Dupree and Hunter, 1983) but are not able to survive temperatures below 15°C (Tave 1990). Tilapia are generally considered herbivorous and detritivorous but will select a diet that maximizes growth (Bowen et al. 1995). Animal protein has been shown to outperform plant protein in the diets of tilapia (Zheng et al. 1988). The dietary needs of tilapia vary depending on species, size of the fish and culture conditions. In intensive culture, the fish must obtain the majority or all of their nutrients from their feed as natural sources such as plants and algae are generally unavailable.

This study was undertaken to determine if the bellies of ocean quahogs could effectively substitute for 100% of the fishmeal in the feed of intensively cultured hybrid tilapia (*O. niloticus* x *O. aureus*) when calcium and phosphorus is supplemented to the diet or if a 9:1 ratio of clam bellies to fishmeal as observed at the Rowland Institute would provide the necessary nutritional balance required for good health and rapid growth.

## MATERIALS AND METHODS

### Experimental diets

Five isonitrogenous and isocaloric diets were formulated to provide 35% protein and 3900 kcal/kg (Table 2). A diet based on herring fishmeal (FM) as the main protein source, at 40.0% of the dry pellet weight, was formulated to serve as the control to evaluate the effectiveness of the clam belly (*Arctica islandica*) diets. Clam bellies were substituted for herring fish meal in experimental treatments at the rate of 50% (HH), 90% (NT) and 100% (CB). Di-calcium phosphate was added to the CB recipe to form a fourth experimental feed referred to as "mineral supplemented" (MS). A secondary control was provided by a commercial tilapia grower feed (ZG) containing 32% protein obtained from Zeigler Brothers, Inc. of Pennsylvania to evaluate overall performance level.

Table 2. Formulations of the experimental diets. The values are percentages based on dry weights.

| | FM | HH | NT | CB | MS |
|---|---|---|---|---|---|
| Herring meal | 40.0 | 20.0 | 4.0 | 0.0 | 0.0 |
| Clam bellies | 0.0 | 20.0 | 36.0 | 40.0 | 39.6 |
| Soybean meal | 11.7 | 11.7 | 11.7 | 11.7 | 11.6 |
| Corn starch | 39.6 | 38.6 | 37.9 | 37.7 | 35.9 |
| Corn oil | 5.7 | 6.7 | 7.4 | 7.6 | 8.0 |
| Gelatin binder | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamin premix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Calcium Di-phosphate | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 |

Fresh minced clam bellies were obtained from Seawatch International of New Bedford, MA, and extruded through a Hobart food grinder. Excess water was removed after weighing to facilitate pellet formation. Fishmeal and/or clam bellies were then blended with the other ingredients composed of soybean meal (11.7%), corn starch (36.2 to 39.6%), corn oil (5.7 to 8.0%), gelatin binder (2.0%) and a vitamin premix (1.0%). Di-calcium phosphate (1.0%) was included in the MS mixture only. All diets were thoroughly mixed and extruded to produce 3 mm pellets which were oven-dried at 82°C for approximately 45 minutes. Pellets were crumbled by a food processor and sifted through a 1 mm screen to remove dust and small particles of food. Pellets were refrigerated in sealed plastic containers until fed to the fish.

**Experimental facilities**

Eight 90 liter capacity fiberglass tanks with individual aquarium-type carbon filters with a flow rate of 1500 liters per hour were used for this trial. Each filter was equipped with two rotating biological contactors (RBC's) for biofiltration purposes. Aeration was continuously provided by air stones. Artificial photoperiod was on a 12L:12D cycle provided by overhead fluorescent lighting. Water quality was monitored routinely. Parameters checked and ranges observed were temperature (26-29°C), pH (6.0-7.5), dissolved oxygen (5.0-6.5 mg/l), ammonia (0-.004 mg/l) and nitrite (0-1.0 mg/l). Water was changed on an as needed basis.

**Experimental Fish and Feeding Protocol**

**Phase I– Comparative Growth Experiment**

Hybrid tilapia (*Oreochromis aureus* x *O. niloticus*) fingerlings were donated by Mr. Jim Tassinari of SEMAP, New Bedford, MA. Two hundred fish were obtained two weeks prior to the start of the trial. Twenty fish were placed in each tank and distributed among the tanks such that each tank had approximately equal starting means and size distributions of fish. During the conditioning period, fish were fed the commercial ZG feed once daily at a rate of 3% of their body weight. At the start of the trial, the fishes ranged in size from 3.3 g to 8.0 g with a mean weight of 5.5 g.

Each of the four feed treatments (FM, HH, CB and ZG) used in the previous study (Stone, 1998) was randomly assigned to a single tank. Each of the newly designed treatments (NT and MS) was randomly assigned to duplicate tanks (Figure 1). Fish were fed to satiation once daily for the first 15 days and then twice daily for the remainder of the trial. Fish were individually weighed every 15 days. This phase of the trial was run for 45 days.

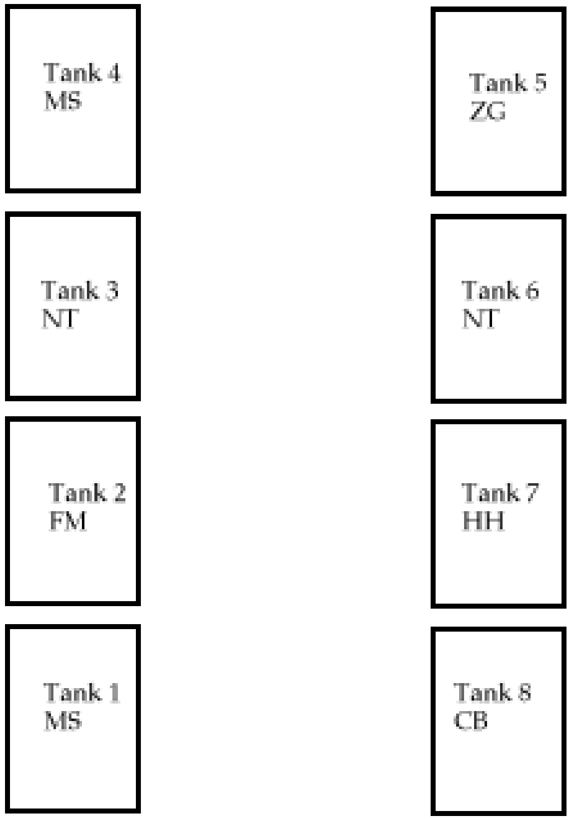


Figure 1. Tank layout and feed assignments for each of the eight groups of fish for Phase I Comparative Growth Experiment.

**Phase II– Feed Switch Experiment**

At the termination of Phase I experiment, half of the fish were culled from each tank based on their size ranking and the ten remaining fish were returned to their tank for Phase II experiment. Size distributions of culled and returned fish were made as equal as possible by lining up the twenty fish by size in individual beakers and pairing them. Of the largest two fish the smaller of the pair was returned to the tank. With the next largest pair, the larger of the two fish was returned to the tank. This alternating selection process was repeated with the remaining pairs.

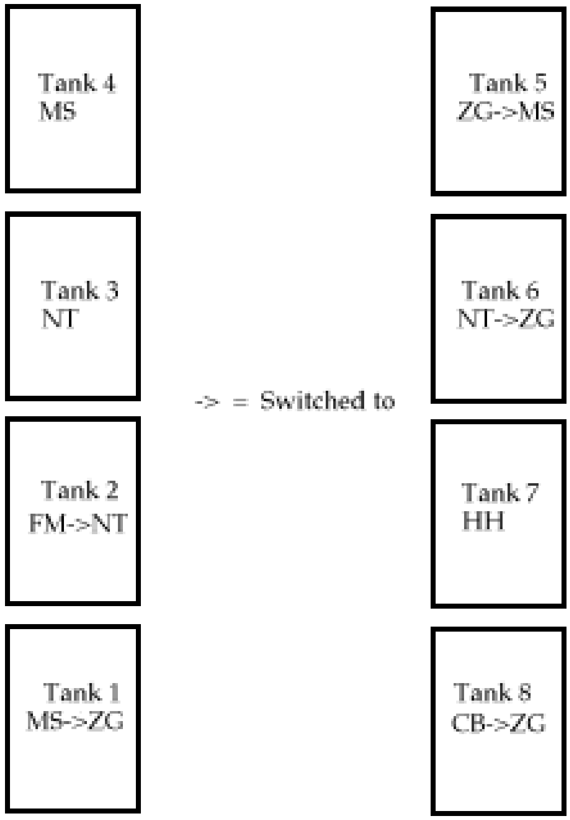


Figure 2. Tank layout and feed assignments for each of the eight groups of

fish during the Phase II Feed Switch Experiment.

Feed treatments in MS1, NT6 and CB8 were switched to Zeigler feed. The feed treatment in FM2 was switched to NT feed and ZG5 was switched to MS feed. The feed treatments in NT3, MS4 and HH7 were not switched and served as controls for Phase II experiment. Fish were fed to satiation twice daily, seven days a week. This phase of the trial was run for 14 days.

**Analysis**

Raw and dehydrated clam bellies were analyzed for proximate and heavy metal composition (Table 3) and amino acid profile (Table 4). The four feed pellet types tested in the previous study (Stone, 1998) were analyzed for amino acid profile (Table 4), proximate composition (Table 5), and mineral content (Table 1). Proximate and amino acid analysis was done by Woodson-Tenent Laboratories of Dayton, OH. Mineral analysis was performed by the ICP Analytical Laboratory at Cornell University.

Based on wet weights of fish and dry weight of feed, apparent and adjusted feed conversion ratios (FCR) were calculated as follows:

Apparent FCR= dry feed fed/ fish wet weight gain

Adjusted FCR= dry feed fed/ (average weight gain * number of fish in each tank at the termination of each phase).

Statistical comparisons were made using one-way analysis of variance. Fisher's PLSD test was used to determine the significance of differences between treatment means at P= 0.05.

Table 3. Proximate and heavy metal analysis

of the raw clam bellies.

| | Wet | Dry |
|---|---|---|
| Moisture % | 85.4 | 0 |

| | | |
|---|---|---|
| Protein % | 11.4 | 78.3 |
| Digestable Protein% | 11.2 | 76.2 |
| Lipid % | 0.7 | 4.7 |
| Crude Fiber % | 0.2 | 1.6 |
| Ash % | 0.5 | 3.4 |
| | | |
| Cd ppm | | 0 |
| Cu ppm | | 10.3 |
| Pb ppm | | 1.3 |
| Hg ppm | | 0.017 |
| Ni ppm | | 1.47 |
| Zn ppm | | 21.2 |
| Cn ppm | | 0.21 |

Table 4. Amino acid profile of raw clam bellies and experimental feed pellets as a percentage of the protein.

| | Raw | Feed Pellets | | | Minimum |
|---|---|---|---|---|---|
| | Clam bellies | CB | HH | FM | Requirements* |
| Amino acid | % of protein | | | | |
| tryptophan | 1.2 | 1.5 | 0.9 | 1.1 | 1.0 |
| lysine | 7.5 | 6.0 | 6.1 | 7.9 | 5.1 |
| leucine | 6.8 | 6.8 | 6.4 | 7.4 | 3.4 |
| isoleucine | 3.6 | 3.9 | 3.4 | 4.2 | 3.1 |
| threonine | 4.7 | 5.0 | 3.9 | 4.6 | 3.8 |
| valine | 4.1 | 4.5 | 4.3 | 4.8 | 2.8 |
| histidine | 2.2 | 2.1 | 2.2 | 3.3 | 1.7 |
| arginine | 8.3 | 6.9 | 5.9 | 6.5 | 4.2 |
| methionine | 4.4 | **0.9** | 1.9 | 2.4 | **me+cy=** |
| cystine | 1.6 | **1.0** | 1.2 | 1.0 | **3.2** |
| phenylalanine | 2.7 | 4.0 | 3.6 | 4.2 | ph+ty= |
| tyrosine | 3.4 | 3.2 | 2.2 | 2.8 | 5.5 |
| glycine | 6.1 | 7.3 | 7.9 | 6.9 | |
| aspartic acid | 11.5 | 11.7 | 9.0 | 10.7 | |
| serine | 5.8 | 5.1 | 4.6 | 4.7 | |

| | | | | |
|---|---|---|---|---|
| glutamic acid | 15.2 | 11.5 | 9.9 | 11.7 |
| proline | 3.8 | 4.8 | 6 | 5 |
| alanine | 6.3 | 5.6 | 5.8 | 6.3 |

*(Santiago and Lovell, 1988)

Table 5. Proximate composition of the experimental feed pellets.

The values are percentages based on the pellet weights.

| | Diet 1 | Diet 2 | Diet 3 | Diet 4 |
|---|---|---|---|---|
| | FM | HH | CB | ZG |
| Moisture | 7.3 | 7.3 | 7.9 | 8.1 |
| Protein | 33.0 | 34.4 | 34.5 | 38.2 |
| Lipid | 10.0 | 8.6 | 8.8 | 11.9 |
| Crude Fiber | 1.2 | 1.0 | 0.8 | 4.0 |
| Ash | 7.4 | 5.5 | 3.2 | 6.0 |
| Carbohydrates | 42.3 | 44.3 | 45.6 | 35.3 |
| Gross energy | 3914 | 3921 | 3998 | 4029 |
| (kcal/Kg) | | | | |

## RESULTS

### Phase I– Comparative Growth Experiment

After 45 days, the fish in FM2 (24.9 g), ZG5 (23.4 g) and HH7 (22.7 g) grew to mean weights which were significantly greater ($P < 0.05$) than the mean weights of the fish in NT3 (17.2 g), MS4 (17.1 g), NT6 (16.1 g), MS1 (16.0 g) and CB8 (14.7) (Table 6). Apparent and adjusted FCR's were lower in the FM2 (App.=1.3; Adj.=1.3), ZG5 (1.4; 1.2) and HH7 (1.4; 1.3) than in the other tanks (App.=1.6–2.0; Adj.=1.5–1.8). The appetite of the fish in FM2, ZG5 and HH7 was considerably better than the fish in the other tanks. Feed intake for those three tanks averaged 460 g per tank which was 33% more than the 346 g average intake of the other five tanks.

The number of mortalities was greatest in ZG5 with three followed by NT3 with two and HH7 and CB8 with one each. There were no mortalities in the other tanks during this phase of the experiment. At the termination of this phase, three fish from the CB8 tank were observed to have deformities in the rostral region consistent with a mineral deficiency. None of the fish in the other tanks were observed to have these deformities.

Table 6. Results of Phase I-Comparative Growth Study (top table) and

Phase II-Feed Switch Experiment (bottom table).

| Phase I-Comparative Growth Experiment |
|---|

| Day 1 | MS1 | FM2 | NT3 | MS4 | ZG5 | NT6 | HH7 | CB8 |
|---|---|---|---|---|---|---|---|---|
| Total weight | 109.4 | 111.9 | 109.4 | 109 | 108.9 | 111.7 | 107 | 107.2 |
| # of fish | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Mean size | 5.5 | 5.6 | 5.5 | 5.5 | 5.4 | 5.6 | 5.4 | 5.4 |
| Day 45 | | | | | | | | |
| Total weight | 320.4 | 498.6 | 309.9 | 342.5 | 397.8 | 322.6 | 431 | 279.5 |
| # of fish | 20 | 20 | 18 | 20 | 17 | 20 | 19 | 19 |
| Mean size | 16.0 | 24.9 | 17.2 | 17.1 | 23.4 | 16.1 | 22.7 | 14.7 |
| Feed given | 329.8 | 520 | 354.7 | 365.2 | 414 | 339.8 | 446.5 | 341 |
| Weight gain | 211 | 386.7 | 200.5 | 233.5 | 288.9 | 210.9 | 324 | 172.3 |
| Apparent FCR | 1.6 | 1.3 | 1.8 | 1.6 | 1.4 | 1.6 | 1.4 | 2.0 |
| Adjusted FCR | 1.6 | 1.3 | 1.5 | 1.6 | 1.2 | 1.6 | 1.3 | 1.8 |

| Phase II Feed Switch Experiment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day 1 | MS-ZG1 | FM-NT2 | NT3 | MS4 | ZG-MS5 | NT-ZG6 | HH7 | CB-ZG8 |
| Total weight | 162.2 | 247.0 | 171.7 | 169.5 | 230.3 | 161.6 | 227.3 | 145.0 |
| # of fish | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Mean size | 16.2 | 24.7 | 17.2 | 17.0 | 23.0 | 16.2 | 22.7 | 14.5 |
| Day 14 | | | | | | | | |
| Total weight | 134.0 | 318.9 | 217.4 | 205.0 | 253.1 | 144.9 | 252.7 | 161.0 |
| # of fish | 6 | 10 | 10 | 10 | 8 | 7 | 8 | 9 |
| Mean size | 22.3 | 31.9 | 21.7 | 20.5 | 31.6 | 20.7 | 31.6 | 17.9 |
| Avg gain | 6.1 | 7.2 | 4.5 | 3.6 | 8.6 | 4.5 | 8.9 | 3.4 |
| Feed given | 56.4 | 92.2 | 58.4 | 63.8 | 77.0 | 55.0 | 80.2 | 47.5 |
| Adjusted FCR | 0.9 | 1.3 | 1.3 | 1.8 | 0.9 | 1.2 | 0.9 | 1.4 |

**Phase II–Feed Switch Experiment**

Fish in FM2 that were switched to NT feed experienced a slight reduction in growth rate when compared to the fish in ZG5 that were switched to MS feed and the fish in HH7 that remained on HH feed (Figure 3). The fish in MS1 and NT6 experienced an increase in growth rate when switched to ZG feed as compared to the fish in MS4 and NT3 which continued with their respective feeds. The fish in CB8 had a small increase in growth rate when switched to ZG feed.

There were a relatively large number of mortalities during this short experiment (Table 6). The greatest number of mortalities occurred in the MS1 tank switched to ZG feed at four followed by the NT6 tank also switched to ZG at three. There were two mortalities in the ZG5 switched to MS feed and in the HH7 tank. One mortality occurred in the CB8 tank switched to ZG feed.

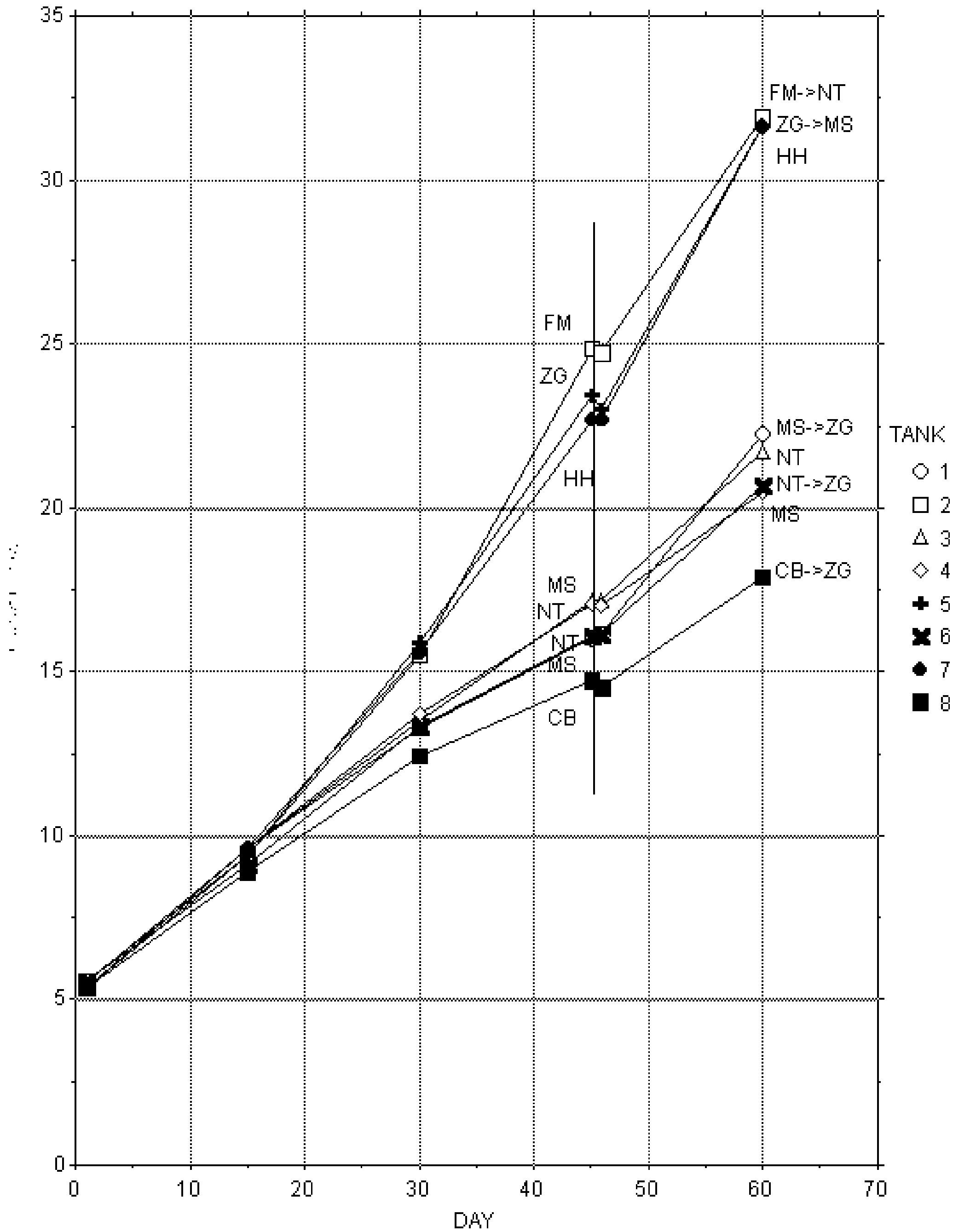


Figure 3. Average growth of fish before (Phase I experiment) and after (Phase II experiment) feeds switched.

## DISCUSSION

A marginal improvement in growth was observed when the clam belly diet was supplemented with di-calcium phosphate (17.4 g and 16.0 g compared to 14.7 g). However, this improvement was not significant ($P > 0.05$) and was significantly less ($P < 0.05$) than fish fed FM (24.9 g), ZG (23.4 g) and HH (22.7 g). This would indicate that a lack of calcium and phosphorus was not the main reason for the poor performance of the CB feed. It was likely the reason for the deformities observed in CB fed fish as three deformed fish were observed in the CB tank and none in the other tanks. The preferred 9:1 ratio of clam bellies to fishmeal that was observed at Rowland Institute did not translate into a suitable feed pellet for tilapia. Average weights of 17.2 g in NT3 and 16.1 g in NT6 were significantly less ($P < 0.05$) than controls.

Proteins are composed of up to twenty amino acids, ten of which are considered essential in the diets of fishes. If any of these essential amino acids are deficient, reductions in growth can occur. The minimum requirement for methionine with cystine is 3.2% of the dietary protein (Santiago and Lovell 1988). Analysis of the raw clam bellies (Table 4) indicated that these two amino acids exceeded minimum requirements (4.4% methionine plus 1.6% cystine equals 6.0% of the protein). But subsequent analysis of the processed CB pellet revealed methionine constituted only 0.9% and cystine 1.0% for a total of 1.9% of the protein. Since the majority of the protein in the CB pellets was derived from clam bellies, this drop in methionine levels would indicate that it was destroyed during the processing of the pellets. Growth of the CB fed fish may be enhanced if methionine supplementation takes place. Shiau et al. (1987) found that the growth of tilapia hybrids was impaired when soybean meal replaced a portion of the fishmeal in their diets, but if the diet was supplemented with methionine, full growth was restored.

Growth in fish is strongly correlated with the amount of feed that they eat. In this study, the fishes that grew best also ate more of the feed. This would indicate that the feeds of the slower eating/growing groups was somehow distasteful to the fish. Amino acids have been used as feeding stimulants for fish. However, the amino acids most used as feeding stimulants (alanine, aspartic acid, glutamic acid, lysine and serine) were not deficient in these feed pellets. Lack of appetite was more likely due to stress induced by the amino acid imbalance caused by the methionine/cystine deficiency.

Further research is needed to determine if clam bellies could be used in a ratio of more than 50:50 but less than 90:10 to achieve their full growth potential or if a clam belly diet supplemented with amino acids would improve appetite and growth.

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**Attachment 4**

**Greater New Bedford Vocational Technical High School**

**Aquaculture-Educational Outreach Program**

**By Rick Brown, Rick Reinecke, and Steve Walker**

A Subsection Of The Project Report: AGRAQUA-298

"Tilapia Aquaculture in Urban "Brownfields":

A Demonstration Project"

December 28, 1998

Prepared for

The Massachusetts Aquaculture Grants Program

Massachusetts Department of Food and Agriculture.

Mr. Scott Soares Coordinator, (617) 727-9800 x 238. Fax: 727-7235.

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With participants:

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5 Fishery Economics Consultants, Fairhaven, MA.

6 Buttonwood Pet Hospital, New Bedford, MA.

7 Rowland Institute of Science, Cambridge, MA.

## INTRODUCTION

### Preliminary Report: March 30, 1998

At the present time, the aquaculture program at Greater New Bedford Voc-Tech High School, in conjunction with the New Bedford Public School's "PALMS" program, has placed 37 tanks in area grammar, junior, and high schools. (list of schools attached)

To begin, a workshop was held to explain the aquarium systems to the teachers, and many other workshops have been held to examine teacher concerns. The only problem we have had is with the town of Fairhaven. They saw an article published in the New England Journal of Medicine dated August, 28th 1997 dealing with *Streptococcus iniae* . The actual conclusion of the six page article is that the people who became infected used a technique described in the article as "this technique contrasts with the methods of purchasing and preparing fish found in many other ethnic communities where the fish are dead before purchase and kept packed in ice in retail stores".

Our conclusion is that the students in the local schools are not in any danger because they do not handle the fish and are not involved in the fillet process. The students in Culinary Arts who do fillet always wear rubber gloves.

During the 2 1/2 years that Voc-Tech has been raising tilapia, we have had 2 fish kills. One was caused by a mechanical failure, and the other was bad judgment. The first, involving about 100 fish, was the failure of a clamp which connects a hot water hose to the heating coil to allow the 150º F water to flow into a 600 gallon tank for about 12 hours. All clamps have been replaced with 100% stainless steel fittings. In the second case, a bucket from Culinary Arts which had contained dish-washing detergent was used to transport fish. There was some residue of soap left in the pail and this caused bacteria on the bio- filter to die. About 30 fish were lost before the problem was rectified.

For the project with UMass Dartmouth and B&D Aquatics, Voc-Tech will host two luncheon workshops and tilapia will be served. Tours of this facility will also be available to interested parties at the conclusion of these workshops.

**Update: December 10, 1998**

As the project at Greater New Bedford Voc-Tech continues, other schools have been added to the list of participants. We are currently working with Lexington Middle school, Essex County Agricultural School, and Minuteman Voc-Tech High School. Also, we have placed five tanks in local schools for the local 4-H organization which will run after school programs using agriculture and hydroponics. We have also placed a tank at the Gidley school in North Dartmouth which was purchased by the school PTA. On Dec. 9, 1998, the parents and students of the fourth grades will have a tour and mini workshop at Voc-Tech. It is obvious the young student interest is being passed on the parents which we feel is an unexpected bonus.

On Tuesday, December 8th, 20 teachers from the New Bedford schools will be at Voc-Tech for an introductory workshop in aquaponics. Joe Winsper (Maintenance teacher) and Rick Brown have designed and built a hydroponics system that will be introduced to 20 local school during the first 2 weeks in January. The purpose of this is to continue our good of educating all local grammar school students in "Aquaponics" (using fish water to grow everything) We intend at the termination of this school year, to give all students a package of vegetable seeds with the hope that they will grow "something" this summer in their yards. New Bedford Voc-Tech also hosted an informational tilapia luncheon in October 1998. Dr. Ibara, Scott Soares, Rick Brown, and Jim Tassinari reported on the up-to-date results of their respective projects. Tours of the Voc-Tech facility are available to any interested parties.

**Preliminary Report: March 30, 1998**

INSTITUTIONS OF LEARNING ASSOCIATED WITH

GREATER NEW BEDFORD REGIONAL VOC–TECH HIGH SCHOOL

IN THE GROWING OF TILAPIA

-

-

**30 GALLON TANKS**

Neighborhood College-UMass Dartmouth Extension, Dartmouth, AM

Atlantic Charter School, Fall River, MA

Gidley School, Dartmouth, MA

Congdon Grammar School, New Bedford, MA

Holy Family Grammar School, New Bedford, MA

Ashley School, New Bedford, MA

Carney Academy, New Bedford, MA

DeValles School, New Bedford,

Duynbar School, New Bedford, MA

Gomes School, New Bedford, MA

Hannigan School, New Bedford, MA

Hayden-McFadden School, New Bedford, MA

Keith Junior High School, New Bedford, MA

Kempton Street School, New Bedford, MA

Mt. Pleasant School, New Bedford, MA

New Bedford High School, New Bedford, MA

Normandin Junior High School, New Bedford, MA

Parker Street School, New Bedford, MA

Phillips Avenue School, New Bedford, MA

Pulaski, School, New Bedford, MA

Roosevelt Junior High School, New Bedford, MA

St. Joseph Grammar School, New Bedford, MA

St. James St. John School, New Bedford, MA

St. Luke's Hospital - Surgery Waiting Room, New Bedford, MA

Jereh Swift School, New Bedford, MA

William HG. Taylor School, New Bedford, MA

Betsy B. Winslow School, New Bedford, MA

Ingraham Adult Ed., New Bedford, MA

Whitman Hanson Regional School District, Whitman, MA

Friends Academy, Dartmouth, MA

St. Mary's Grammar School, New Bedford, MA

**400 GALLON TANK**

Wareham High School, Wareham, MA

**600 GALLON TANK**

Diman Regional Vocational High School, Fall River, MA

**Attachment 5**

**A Brief Review of Tilapia Reproduction and**

**Preliminary Fry Production Experiments with**

**Hybrid Tilapia (*Oreochromis niloticus x O. aureus*)**

**By Karen Zimmerman, Richard Brown, Adam Magdy and Richard M. Ibara**

A Subsection Of The Project Report: AGRAQUA-298

"Tilapia Aquaculture in Urban "Brownfields":

A Demonstration Project"

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## A Brief Review of Tilapia Reproduction

**Introduction**

There are three major advantages for a grower to produce his/her own fry: cost reduction, disease control, and genetic selection. While tilapia readily breed in captivity, certain biological and environmental factors significantly affect the quantity and quality of fry produced.

**Cost Reduction**

One of the advantages of raising tilapia in aquaculture is that fry are readily available throughout the year from commercial hatcheries. In volume, fry average about 10 cents each delivered (Georgianna 1998). At full grow-out capacity about a million fry per year costing $100,000 are needed. At this point, it probably is economical for a tilapia farmer to produce his/her own fry.

The cost of production could be lowered if tilapia were bred "in house," by reducing the cost per fry and by better controlling uniformity in the size of fry. Once a fry production system is worked out cost per fry probably could be lowered from 10 cents to three to six cents per fry. Maintaining a uniform size of fish in the grow out tanks helps to reduce food competition and eases manageability of stocks (Balarin and Haller 1982) further reducing production costs.

**Disease Control**

The threat of introducing diseases from another aquaculture facility is very real. As a biosecurity measure, the North Carolina State University tilapia demonstration project requires visitors coming from another facility to shower and change into fresh clothes before they are allowed to visit (Lorsodo, Tom, pers. comm.). One of the largest tilapia hatcheries in the U.S. advertises *Streptococcous iniae,* free fry. This rare bacterium may not be fatal to tilapia but individuals stabbed by the spines of an infected fish may require hospitalization. There were a few cases of infected individuals in Montreal in 1997. Obviously, a facility maintaining their own broodstock and producing their own fry lessen the chances of introducing new diseases.

**Genetic Selection**

By controlling the breeding of broodstock, certain desirable characteristics, such as all-male brood, increased size, faster growth rate, and thicker body width can be selected (Balarin and Haller 1982). Fish possessing these characteristics need to be selected, isolated and maintained as broodstock. Careful records need be kept.

**All-Male Broods**

Because male tilapia have higher growth rates and better food conversion ratios, monosex male culture has obvious advantages (Guerrero 1982, Landau 1992, Stickney 1979). Further, in a mixed population, uncontrolled reproduction is likely to occur resulting in over population and growth stunting (Jalbert and Zohar 1982; Nogueira da Silva et al. 1997). A monosex all-male culture can be produced either by manual separation of the sexes, by hormonal treatment at a critical larval stage, or by hybridization of XY and WZ species. A disadvantage of the latter two methods of producing monosex broods is the inability to use the progeny for future broodstock, so that additional tanks of broodstock must be maintained.

**All-Male Brood by Hybridization**

Various hybrid crosses of Tilapia species result in all or nearly all male F1 generation (Guerrero 1982, Landau 1992, Stickney 1979). Explanations for this are linked to the sex-determining mechanisms of XY and WZ species (Guerrero 1982, Lovshin 1982). In the XY system of sex determination, males are the heterogametic sex (XY) and females are homogametic (XX). In the WZ system, the opposite is true. Females are heterogametic (WZ) and males are homogametic (ZZ). By crossing an XX female from one species with a ZZ male of another, theoretically all offspring would be XZ. Since the male Z gene is assumed to be dominant to the female X gene, all offspring should be male (Tave 1987). Lovshin (1982) believes inconsistencies predicted by Mendelian genetics in the sex-ratios of some hybrid crosses can be explained by contamination of the "pure" broodstock lines. The effects of autosomes as well as sex chromosomes have been shown to influence the sex ratio of progeny (Guerrero 1982, Mair et. al 1997). Examples of hybrid crosses favoring male offspring are shown in Table 1.

Mair et. al (1997) were able to produce YY males as compared to the normal XY males of *Oreochromis niloticus* and *O. mossambicus*. When these are crossed with normal XX females, all offspring are XY genotypes and an average of 96% males are produced.

Table 1. Examples of Hybrid crosses that yield high percentages of males in the

F1 generation. (Note: The genus of the species listed in this table is now *Oreochromis*.)

| Male | Female | source | % Male produced |
|---|---|---|---|
| *Sarotherodon hornorum* | *S. spilurus niger* | Guerrero 1982 | 100 |
| *S. variabilis* | *S. niloticus* | Guerrero 1982 | 100 |
| *S. leucostictus* | *S. niloticus* | Guerrero 1982 | 93-98 |
| *S. spilurus niger* | *S. niloticus* | Guerrero 1982 | 93-98 |
| *Tilapia hornorum* | *T. mossambica* | Landau 1992, Guerrero 1982 | 100 |
| *T. aurea* | *T. nilotica* | Landau 1992, Stickney 1979, Guerrero 1982, Lovshin 1982 | 100 |
| *T. macrochir* | *T. nilotica* | Landau 1992, Guerrero 1982 | 100 |
| *T. mossambica* | *T. nilotica* | Landau 1992 | 100 |
| *T. hornorum* | *T. nilotica* | Landau 1992, Guerrero 1982 | 100 |
| *T. aurea* | *T. vulcani* | Landau 1992 | mostly |
| *T. aurea* | *T. mossambica* | Stickney 1979 | not available |

### All-Male Brood by Sex Reversal

Hormonal treatment of fry can also result in monosex broods. Fry can be treated with androgenic steroids to induce the sex reversal of genotypic females within a brood (Guerrero 1982, Balarin and Haller 1982, and Rosati et al. 1997, Watanabe 1997, Stickney 1979). Typically, fry are fed a diet treated with 60 g/ ml of 17 a- ethynyltestosterone for three to six weeks when fry reach a length of 9-11 mm (Guerrero 1982, Rosati et al. 1997, Stickney 1979, Teichert-Coddington and Green 1997, Watanabe 1997). Also proven to be effective in sex reversing fry is methyltestosterone in the same dosage, but the use of this drug has not yet been approved for use in feeds for fish intended for food consumption in the U.S. (Guerrero 1982, Watanabe 1997).

### Reproductive Behavior

In nature, male fish dig a spawning bower and defend it against other males. The bower differs among species, but the most common is a depression dug in the sand and kept clear of debris. The female enters the bower and forms a temporary pair bond with the male. Spawning can take from 45 minutes to two hours. The female releases her eggs in batches of 20 to 50 while the male deposits sperm on them. The female then picks the eggs up in her mouth and incubates them. After spawning the female leaves the bower. The males are polygynous. Depending on the size and age of the female, a few hundred to a few thousand eggs are produced (Rana 1988). Even after hatching, the larvae remain in the mouth and continue to return for protection after they are free swimming fry (Fitzsimmons 1997a, Rana 1988).

### Age, Size and Fecundity

In most cases, tilapia reach sexual maturity in less than six months (Fitzsimmons 1997a). However, under certain conditions of light, temperature, food and social factors, sexual maturity can be reached as early as three months (Jalbert and Zohar 1982, and Balarin and Haller 1982). For example, inadequate food supplies have been shown to promote early maturation with lowered fecundity and stunted fry. This is assumed to be an adaptation for species survival during food limited periods (Balarin and Haller 1988). When food supply is good both in quantity and quality, fry production is stimulated. Broodstock fish require a high protein diet. Fish fed a diet of 35-40% protein produce the most fry (Balarin and Haller 1988).

Once mature, spawning can occur every 10-14 days, with females producing an average of nearly 600 eggs and fry per spawn depending on size and age at the time of spawning (Rosati, et. al 1997).

### Photoperiod and Reproduction

Extended photoperiods and strong illumination have been shown to both stimulate and enhance breeding activity. However, these same conditions have also been shown to be stressful and delay sexual maturation in developing fry (Balarin and Haller 1982). The length of the photoperiod affects breeding behavior as was shown in the work of Baroiller et al. (1997). They demonstrated that when the Light:Dark ratio was 8L:16D no breeding took place, but when the ratio was increased to imitate the natural changing of seasons toward summer light conditions, breeding occurred. The number of fry produced increased steadily with increased light until a maximum of approximately 14L:10D. After that, increased light availability did not result in increased fry production.

Extended day lengths of 16L:8D and 24L:0D have been found to inhibit growth in young fish(Watanabe et al. 1997).

**Temperature and Reproduction**

Watanabe et al. (1997) has suggested that water temperature plays a more dominant role in stimulating gonadal development that does photoperiod. They found that increasing temperature alone were able to induced spawning in a number of tilapia species. Balarin and Haller (1988) reported that when temperature was reduced from $25^0$ to $18^0$C for two weeks and then increased to $25^0$C, greater than 50% of their females spawned. Temperatures above $20\text{-}23^0$C are required for spawning and for the growth of secondary sexual characteristics. Prolonged temperatures below $20^0$C results in the failure of juveniles to mature and in the reabsorption of mature gonads in older fish (Balarin and Haller 1988, Jalbert and Zohar 1982).

**Environmental Complexity and Reproduction**

Environmental complexity stimulated both the reproductive activity and fry survival rates in Tilapia. By placing objects into the race ways, males more readily established breeding territories and nests and young fry were better able to avoid predation and cannibalism (Baroiller et al. 1997).

**Broodstock Density, Sex Ratio and Reproduction**

Over stocking of tanks results in little or no fry produced as there is too much competition for breeding sites, and too much predation on eggs and fry. Stocking densities over 10 kg/$m^3$ have resulted in no successful spawning. The sex ratio of the broodstock is also of great importance. Generally a ratio of 3-5 females for every male is used (Fitzsimmons 1997a, Rosati et al. 1997, Balarin and Haller 1988, Mires 1982, Baroiller et al. 1997, Paessun and Allison 1984). In cases where the ratio was 1 to 1 there were more fry per female produced, but production is in bursts, not continuous. When the ratio was raised to 2 females to 1 male more fry were observed, but the ratio of 3 to 1 yields a more continuous production of fry and more total fry produced (Guerrero 1982). Fry production decreases with ratios greater than 4 to 1. Rana (1988) found that when the sex ratio was changed from 4 to 1 to 3 to 1 the interspawning interval was reduced from 35-49 days to only 28-40 days.

**Dominance Hierarchy and Reproduction**

A dominance hierarchy has been shown to develop in at least two species of *Oreochromis*. This hierarchy results in certain dominant females who produce most of the fry. It appears that subdominant females only spawn after dominant females are already brooding. If this is the case, and eggs and larvae are harvested every two weeks by shaking them loose from the mouth of brooding females, it will tend to be the alpha and beta females that will have seeded at each collection. Also the seed harvested from the alpha females will be a few days further developed than that collected from beta females (Baroiller, et. al. 1997).

**Broodstock Conditioning**

If fish were to reproduce every two weeks for a long period of time, production would drop off as the females became exhausted. Resting of the broodstock is a common practice to prevent this. Fish are allowed to remain in the brooding tanks until production begins to drop in about three months. They are then moved to a conditioning tank to rest for a period of about six months. To maintain a continuous production of fry three sets of broodstock must be maintained (Rosati, et. al 1997).

**Methods of Harvesting Fry**

Perhaps the largest threat to eggs, larvae, and young fry is cannibalism by their tank mates. In a study done by Berrios- Hernandez (1983) fry were either left with their parents in the breeding tanks, removed as eggs, or removed as free swimming larvae. He found no significant difference in production between removing fry or eggs from the adults, but leaving fry with adults resulted in only 2-3% of the harvest as compared with tanks from which fry were removed. Because fry are so readily consumed by conspecifics it is important to establish a method of harvesting fry. Also Rana (1988) reports that removing the fry from incubating females reduced the interspawning period by half. Harvesting of the fry can be done in many different ways.

**Hapa Net Method**

A hapa net is a fine mesh net hung on a frame in a body of water. The adults can be removed by lifting a thicker mesh net pre-hung inside the hapa net. Young fry can be seined out of the tank in which they were hatched and placed into these hapa nets where they can be fed without threat from adults (Fitzsimmons 1997a). This method does not allow for the hormonal treatments to reverse the sex of the fish.

**Spawning Tank Method**

In hatcheries where selective breeding is taking place, the brood fish selected are placed into a spawning tank (for example, see Rosati, et al. 1997). From here the fry can either be seined out as soon as they are free swimming, or the eggs and larvae shaken from the mouths of the females and placed into separate rearing tanks (Fitzsimmons 1997a, Le Coz et al. 1990, Paesseun and Allison 1984).

Removing all adults from a spawning tank after two to three weeks and leaving the fry to grow in the spawning tank is another method of separating adults and fry. Rosati et al. (1997) reports their practice of removing adults only long enough to catch the fry for transfer to a nursery tank. The buccal cavities of the females are searched for additional eggs as they are returned to the spawning tank. This is done every 10 -14 days.

**Rearing of Fry**

When brooding, the female continuously rolls and churns the eggs within the buccal cavity. Some aquaculturists have tried using both air and water to agitate the eggs and larvae. Conical upwelling containers and shaking tables seem to be the most common devices used (Rana 1988, Fitzsimmons 1997). McDonald jars are another popular device for the rearing of fry (Rosati et al. 1997). When fry become 20 g fingerlings after about 12 weeks, they are either sold or moved to grow out tanks.

## Preliminary Fry Production Experiments with Hybrid Tilapia (*Oreochromis niloticus x O. aureus*)

**Abstract**

Two experiments were conducted, one to test a mass fry production technique and the other to test the effect of water clarity on breeding success of hybrid tilapia which use visual display in courtship behavior. A 600 gallon tank with 20 fish (presumed female to male ratio of 3:1) was used to test the former objective and two 40 gallon aquaria, one with clear water and the other with colored water were used to test the latter objective. Each of the two aquaria contained three females and one male. Fish were monitored daily for signs of breeding behavior for nine weeks. The experiments were conducted at G.N.B.R. Voc-Tech High School.

After six weeks, a single fry was produced. No additional fry or eggs were noticed in the last three weeks. After the ninth week, 26 of the 28 fish were sacrificed and sexed. There were 11 females and 15 males as compared to the presumed 20 females and 6 males (ratio of 3 to 1).

**Introduction**

Tilapia are fecund species and readily reproduce in captivity (Jalbert and Zohar 1982, and McLarney 1987). As such, fry are readily available throughout the year from commercial hatcheries and, in quantity, sex-reversed all-male fry average about 10 cents each delivered (Georgianna 1998). Hence, there is not an urgent need for a tilapia farmer to produce his/her own fry. However, at full grow-out capacity, about a million fry per year costing about $100,000 are needed. At this rate of production, an opportunity exists to reduce fry production cost. Other advantages for a farmer to produce his/her own fry include the opportunity to genetically select certain traits and to reduce the chance of importing diseased fish and contaminating the entire facility.

Several biological and environmental factors affect reproduction in tilapia. Sex ratios, stocking density, water temperature, photoperiod, water quality, food quality and quantity, pollutants and stress are among the known factors affecting fry production rates (Baroiller et al. 1997). To gain experience and test a technique in mass producing fry and to test whether water clarity has any effect on breeding success of hybrid tilapia which use visual display in courtship, preliminary experiments were conducted at G.N.B.R. Voc-Tech High School.

**Materials and Methods**

A 600 gallon tank was used to test a technique of mass producing fry. Twenty fish consisting of 5 males and 15 females (judged by the shape of the posterior edge of the dorsal fin) were placed in the tank. Several short pieces of PVC pipe were placed in the tank to provide territorial markers for the males.

To test whether water clarity might affect breeding success of hybrid tilapia two 40 gallon aquaria were used. While both aquaria were 40 gallon tanks, one aquarium was taller and narrower (40H) than the other (40L). Each tank was initially stocked with one male and three females. The 40H tank was treated with blue food coloring to partially obscure the vision of the fish. Pieces of PVC pipe were placed in the 40H tank only. After six weeks the water was treated with additional food coloring to further decrease visibility; the PVC pipes were removed at this time.

Hybrid tilapia (*Oreochromis niloticus x O. aureus*) approximately six months of age were used. The fish were obtained from Voc-Tech and probably originated from B&D Aquatics.

The temperature of the three tanks was maintained at 85-90$^{O}$F. For the first six weeks the photoperiod of the overhead low-output florescent lights was set at 12L:12D. subsequently the lights were set at 16L:8D. For the first two weeks the fish were not fed. Following that, they were fed Ziegler brand floating pellets once daily.

**Results**

Only a single fry resulted from this effort. This fry was found in the 600 gallon tank after about 20 days. No other fry were produced during the experimental breeding program. An examination of the broodstock showed that four females were ripe and had well developed ovaries and two of the males had reasonably large testes. On average males were 3.2 cm longer than the females, yet had a gonosomatic index (ratio of gonadal weight over body weight times 100) only 6% of that of the females (Table 1a and 1b).

Table 1a.

The weight (g), standard length (mm), gonadal weight (g), and

gonosomatic index of the males.

| | | | | | |
|---|---|---|---|---|---|
| | **No.** | **Wt (g)** | **Std Ln (mm)** | **Gonad Wt (g)** | **GSI** |
| | 1 | 78 | 115 | 0.05 | 0.06 |
| | 2 | 132 | 151 | 0.05 | 0.04 |
| | 3 | 139 | 156 | 0.20 | 0.14 |
| | 4 | 164 | 137 | 0.30 | 0.18 |
| | 5 | 230 | 164 | 0.30 | 0.13 |
| | 6 | 180 | 167 | 0.30 | 0.17 |
| | 7 | 173 | 203 | 0.39 | 0.23 |
| | 8 | 218 | 150 | 0.40 | 0.18 |
| | 9 | 194 | 164 | 0.43 | 0.22 |
| | 10 | 142 | 158 | 0.49 | 0.35 |
| | 11 | 161 | 160 | 0.49 | 0.30 |
| | 12 | 239 | 177 | 0.54 | 0.23 |
| | 13 | 207 | 181 | 0.67 | 0.32 |
| | 14 | 199 | 180 | 0.90 | 0.45 |
| | 15 | 182 | 165 | 0.91 | 0.50 |
| | **MEAN** | **176** | **162** | **0.43** | **0.23** |

Table 1b.

The weight (g), standard length (mm), gonadal weight (g), and gonosomatic index of the females.

| | [redacted] | | | | |
|---|---|---|---|---|---|
| | **No.** | **Wt (g)** | **Std Ln (mm)** | **Gonad Wt (g)** | **GSI** |
| | 1 | 135 | 104 | 0.60 | 0.44 |
| | 2 | 57 | 113 | 0.11 | 0.19 |
| | 3 | 102 | 115 | 0.20 | 0.20 |
| | 4 | 116 | 140 | 0.32 | 0.28 |
| | 5 | 112 | 146 | 0.78 | 0.70 |
| | 6 | 51 | 115 | 0.86 | 1.70 |
| | 7 | 157 | 153 | 1.90 | 1.21 |
| | 8 | 111 | 129 | 1.98 | 1.79 |
| | 9 | 159 | 157 | 2.80 | 1.76 |
| | 10 | 142 | 133 | 3.04 | 2.14 |
| | 11 | 88 | 130 | 27.70 | 31.48 |
| | **MEAN** | **112** | **130** | **3.66** | **3.81** |

### Discussion

This nearly total failure in producing fry was unexpected. Two years ago B&D Aquatics experimented in breeding tilapia in a 50 gallon aquarium. The tank was stocked with two males and eight females. Every two weeks eggs were shaken out of the mouths of brooding females resulting in about ten thousand fry produced in the course of a summer. Thereafter fry production slowed considerably. We expected similar success.

It is difficult to determine why the fish did not breed. Sexual maturation occurs as young as 3 months of age (Jalbert and Zohar 1982). The fish used were six months of age at the start of the experiment. Fitzsimmons (1997a) reports that adults are sexually mature in less than six months. This leads us to believe that the age of the fish was appropriate.

The assumption that the ratio of females to males in this experiment was similar to the stocking ratio of three to four females per male recommended by Fitzsimmons (1997a) was erroneous. A final dissection of the fish showed that there were only eleven females and fifteen males. While this ratio is inappropriate and should cause reduced amounts of fry, it does not explain the nearly complete failure to reproduce.

Environmental complexity has been shown to increase fry production, both in terms of fry survival and reproductive activity (Baroiller et al. 1997). The addition of PVC pipe sections in the 600 gallon tank provided for this.

Water temperature is known to have a significant effect on reproduction. Low temperatures of 15°C to 22°C inhibit reproduction completely (Jalbert and Zohar 1982). Watanabe et al. (1997) suggest that water temperature plays a more dominant role than photoperiod in stimulating gonadal production. In this experiment a failure of a heater in the large aquarium resulted in the water temperature dropping for a period of 2-4 days during the first week of set up. This was probably not significant as the low temperature did not persist for an extended period of time and was corrected within the first week of study.

A possible reason for the failure is that the fish used may have been hormonally sex-reversed hybrids. While sex-reversal is typically only 80 to 99% successful, the treatment itself might affect the behavior and physiology of the phenotypic males and females. Further, some hybrid species do not breed as well as others. The fish we used were probably hybridized for many generations, and their genetic strain is largely unknown.

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**Attachment 6**

**Evaluation of Disease Software**

**By Adam Magdy**

A Subsection Of The Project Report: AGRAQUA-298

"Tilapia Aquaculture in Urban "Brownfields":

A Demonstration Project"

December 28, 1998

Prepared for

The Massachusetts Aquaculture Grants Program

Massachusetts Department of Food and Agriculture.

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**Evaluation of Fish Disease software**

Two disease software packages were evaluated. Fish Vet 2 (from Fish Vet Inc. with U.S. address of 12620 Ivy Mill Road, Reisterstown, MD 21136) costs $999 and comes on CD, while the Hawaiian Aquaculture Multiple Expert System (HAMES) from the University of Hawaii Extension Service is available free through the internet at the American Tilapia Association website. Fish Vet 2 has graphic interactive features and is extensive in coverage but falls short of its advertised promises and is not very useful for the tilapia farmer. The program is protected by a hardware device that attaches to the printer port. HAMES is a text-based system and offers practical suggestions to problems based on information supplied by the user. HAMES can actually be quite useful. See Attachment 6, Evaluation of Disease Software.

**I . FISH VET SOFTWARE**

Expert system based software deals with bacterial, viral and parasitic diseases in both fresh and saltwater fish. The system is a multimedia based program that contains large quantities of digitized color photographs of signs and diseases as well as abnormal observations. This is the basic concept of the software as the selling n advertising brochures describe the features of Fish Vet.

In theory, the Expert System of databases of different diseases n then a diagnosis based on choosing different signs is a sound approach. But in application, the program fails in delivering a good value to the fish farmer. When you consider the number of fish and the number of diseases each fish is exposed to, the scope of the program is very wide. Consequently, the depth of information in each category is not as complete a problem solving tool as claimed by the producer of the software.

The software is based on a Windows 95 platform. It is easy to use and install in general although the hardware protection device makes it somewhat difficult to print the results using a printer. The program is composed of four sections:

**Disease browsing** :

A list of is given of 108 different common diseases of salt and fresh water fishes, their signs and signs explanation. The descriptions include causes, epidemiology, prognosis, treatment and short essay. In this section, there is a space for contributors and anther space for adding a user notice.

**Diagnosis Section**:

By using a list of different common signs listed by the program, one might arrive a probable diagnosis of a disease

**Bio Profile Section**:

By selecting a biochemical profile of the bacteria isolated one might reach the bacterial identification of the causing organism

**Parasitic Section:**

Seventy two different color pictures of common fish parasites are given. By identifying them one can reach a possible diagnosis of a parasite infestation.

In general, the coverage of the commercially important fish diseases is rather shallow. Considering the scope of the subject it is my hope the future issues of the software will be able to ovecome the shortcomings of the present version.

**II. HAMES SOFTWARE Hawaii Aquaculture Multiple Expert System)**

This software is sponsored by United States Department of Agriculture through the Center of Tropical and Subtropical Aquaculture of the University of Hawaii. It can be downloaded for no charge at the American Tilapia Society web page. It is directed to the tilapia fish farmer to solve everyday problems

The software is available in the Windows 95 environment. It is very easy to use and it is quite helpful for the fish farmer in solvig everyday problems especially those related to water quality and common bacterial and parasitic problems. The program is divided into the following sections:

**1. How to use HAMES** Overview of Hames, what it can and can't do and a general action to accomplish tasks.

**2. Reading Topics** Use to select a reading topic from a list of aquaculture topics related to HAMES.

**3. Glossary** This section describes the most common scientific and technical terms that are used in HAMES.

**4. LABORATORY METHODES HAMES** Requires a specific operation for the diagnosis of problems and treatment. This section provides detailed information on how to accomplish these activities.

**5. BIBLOGRAPHY** Here you can look at an extensive list of publications related to HAMES. It can also be printed.

**6. EXENSION ASSISTANT** List of name and addresses of personnel who might be of assistance.

**7. VENDORS** List of names and addresses of vendors who can supply materials mentioned in HAMES.

In application the program proves itself to be very practical for the tilapia fish farmer and its' rather simple approach is an asset when it comes to solving everyday problems. In contrast to FISH VET, HAMES will win when it comes to Tilapia Aquaculture

**Attachment 7**

**Taste Discrimination Experiments at Rowland Institute of Science**

**By Jean Fraser, Ava Chase and Wynfield Hill**

A Subsection Of The Project Report: AGRAQUA-298

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**ABSTRACT**

Previous work at the Rowland Institute has shown that an adult tilapia will select and thrive on feed with nine parts of the protein based on clam bellies and one part based on fishmeal. However, at UMass, fingerlings fed according to this ratio did not do well. We attempted to test fingerling tilapia using the same experimental choice paradigm used on adult fish. We asked the question: Would the fingerlings pick a different ratio than the adult fish? If we could answer this question then we would have a fast means of developing feed for animals as they matured. We have, so far met with very little success. The small fingerlings (1-2 inch) were impossible to train and the equipment, built for larger fish, was too insensitive. We moved to slightly larger fish (about 4 inches) and redesigned the equipment to make it more sensitive and the task easier to learn. Even with the redesigned equipment the young fish are difficult. They appear to be more apt to develop a side bias and are more distractible. The results are

inconsistent but there appears to be a trend to prefer a diet higher in fishmeal. We believe this method of determining fish diet is very promising, but it clearly needs more work.

**BACKGROUND**

Although shellfish aquaculture is an established industry in the northeast, finfish aquaculture is in its fledgling stage and still struggling to be economically viable.

The single greatest expense for an aquaculturist is feed and the most expensive ingredient in feed is fishmeal. A nutritious, palatable, inexpensive fishmeal substitute could significantly lower the cost of finfish aquaculture in the Northeast.

The ultimate goal of the studies reported here is to find a low-cost substitute for fishmeal in aquaculture feeds. To this end we have examined several local sources of protein that are waste products of the seafood processing industry. The most promising of these is clam bellies a waste product of the clam industry. At this time, the disposal of this waste represents a significant cost to the processor and a potential pollutant to the environment.

An analysis of clam bellies shows a complete amino acid profile and a high protein level. In addition, studies at UMass Dartmouth and at Voc-Tech have shown that although fish fed a formulation with all the protein supplied by clambellies do not grow well, fish fed a formulation with the protein supplied by half fishmeal and half clam belly grow as well as those on commercial feed. We concluded that, given the two variables - clam belly and fishmeal - the fish needed at least some fishmeal in their diet to remain healthy. Just how much clam bellies the fish could tolerate was our next question..

**Rowland experiments**

At the Rowland Institute for Science in Cambridge MA. we used an experimental set up designed for cognitive studies on fish to test food preferences in tilapia.

The tilapia used in the experiment were adults that had been raised on commercial food and had never experienced the experimental formulations.

The fish were trained to press a button to receive a food pellet. They were then given a choice between two formulations - the same ones that were used in the Voc-Tech and UMass experiments. One button would give them a pellet that was clam belly based and the other gave them a pellet that was fish meal based. The tilapia could take as much of either or both of the formulations as they wanted. Sessions lasted an hour a day and the experiment ran for 50 days. This study demonstrated that adult tilapia would consistently select a diet of nine parts clam belly to one part fishmeal and thrive.

**BROWNFIELDS STUDY**

Based on the previous studies, we asked the question – Could the different results from the UMass-Voc-Tech study and the Rowland study be a function of the different ages of the tilapia in the two experiments?

We obtained fingerlings from Voc-Tech and attempted to replicate the choice experiment with much younger animals.

We have so far, met with little success with the smaller animals. The fingerlings are extremely difficult, if not impossible, to train. The equipment built for larger fish was not sensitive enough to record button presses of the fingerlings.

We moved to slightly larger fish and redesigned the equipment to make it more sensitive and the task easier to learn.

FIGURE I

Operant apparatus - fish button

INSERT by scanning fig.

Even with the redesigned equipment the young fish are difficult. They appear to be more apt to develop a side bias and are more distractible. The results are not significant but there appears to be a trend to prefer a diet higher in fishmeal protein than do adult fish.

We believe this method of determining fish diet to be very promising but it clearly needs more work.

## List of Photographs with Captions

### Attachment A'. PHOTOS SUBMITTED WITH PRELIMINARY REPORT

Photo A1. GNBR Voc Tech HS room devoted to reproductive experiments.

The 600 gallon tank is in the foreground with the aquaria in the back. Jean Fraser, Steve Walker (left), and Rick Brown.

Photo A2. GNBR Voc Tech HS aquaculture facility. Visitors are first and third graders from William H.G. Taylor School, New Bedford, MA. The teachers are Mrs. Zajac and Mrs. Vargo hosted by Mr. Scott Davidgnon and Mr. Rick Brown.

Photo A3. Feed trials at UMass Dartmouth. The pink color of the fish in Tank 1, most notable at the base of the pectoral fin, caudal fin, and the dorsal fin (very apparent in Photo C5), is typical of nearly all 100 fish fed high clam belly diets.

Photo A4. Tanks 5 to 8 of UMass Dartmouth's feed trial experiment.

Photo A5. The south half of the second floor at B & D Aquatics.

This was the condition of the north half prior to clearing and cleaning.

Photo A6. The RBC with the cover off and the screen filter for the second floor Tank 2A (right silhouette) and Tank 2B (left silhouette).

Photo A7. Newly installed second floor tanks. Tank 2B is in the foreground and Tank 2A is in the background with the RBC between them.

Photo A8. Acknowledgment to the Massachusetts Department of Food and Agriculture for their support. Two signs are posted, one on the first floor and the other on the second floor. These signs will be posted for at least three years.

Photo A9. First floor circular grow-out Tank A with the RBC in the background.

Photo A10. The filter system for Tank A. The RBC is in the foreground, the screen filter is in back of it, and the protein skimmer is to the left.

Photo A11. Newly installed first floor 10,000 gallon Tanks F, G (right background) and H with the two biological filter tanks in the middle of the grow-out tanks.

Photo A12. Newly installed Tank F with its RBC filter.

Photo A13. Part of the 5,000 fish in the 10,000 gallon grow-out Tank A.

Photo A14. First floor rectangular Tank B (left) and Tank C.

The oxygen injection system for Tank C is in the foreground.

### Attachment B'. PHOTOS OF TANK SYSTEM AT SEMAP

### (FORMERLY B & D AQUATICS) SUBMITTED WITH FEASIBILITY REPORT

Photo B1. First floor – 10,000 gallon tanks A, E, and FII. Tanks A, far rear, and E, center, are on separate RBC filters (green structures to their left). Tanks FII, front left, and FI (not visible) are filtered by a common system.

Photo B2. First floor – 10,000 gallon tanks E, FI and FII.

Tank E, foreground, is an independent system, while tanks FI, rear left, and FII, rear right, are linked by the blue filters between them.

Photo B3. Second floor – 5,000 gallon tanks, GI and GII. Tank GI, foreground, and Tank GII, barely visible in the background, are connected to a common filter system, center left. This system was funded by a grant, Agraqua-298, from Mass DFA.

Photo B4. First floor – 5,000 gallon tanks, C and B.

Each tank, C, left, and B, right, has its own filter system at the far end. The tanks are 50 feet long. The large pipe in the center left is the oxygen injection system.

**Attachment C'. NEW PHOTOS SUBMITTED WITH THIS FINAL REPORT**

Photo C1. B & D Aquatics (Now SEMAP) site.

Photo C2. Vacant mill space at Brook and Deane Street.

Photo C3. Second floor – 5,000 gallon tanks, GI and GII with RBC and screen filters.

Photo C4. Second floor – 5,000 gallon tank, GII with insert of RBC and screen filters.

Photo C5. Juvenile tilapia at UMass fed clam belly pellets supplemented with Ca & P.

Notice the pink color of the fish, most notable at the base of the pectoral fin, caudal fin, dorsal fin, and snout. This condition may be due to ascorbic acid deficiency.

Photo C6. Juvenile tilapia at UMass fed fishmeal pellets. Notice the normal coloration in contrast to C5 fish. All of the pellets formulated were fortified with vitamins but ascorbic acid may have been denatured in drying the clam belly pellets.

Photo C7. First flood biological filter vats for tanks FI and FII.

Photo C8. Close-up of fiber bed of above vats, left, and close-up of fiber filter for tanks B and C.

Photo C9. Aquaculture tank and hydroponics system developed by Voc-Tech.

Photo C10. Two of Voc-Tech's 500-gallon tank systems. The blue trays are the solid removal system and the floating cylinders are the RBC filters.